

ORIGINAL ARTICLE: CLINICAL

## Denileukin diftitox for the treatment of CD25 low-expression mycosis fungoides and Sézary syndrome

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### Abstract

In a placebo-controlled study, denileukin diftitox (DD) was effective against cutaneous T-cell lymphoma (CTCL) expressing CD25. An open-label companion study examined the efficacy and safety of DD in 36 patients with skin biopsies containing <20% CD25 cells by immunohistochemistry staining (CD25 low expression). Patients received DD 18 µg/kg/day for 5 consecutive days every 3 weeks for up to eight courses. The primary endpoint, overall response rate, was 30.6% (95% confidence interval: 16.3, 48.1), 33.3% for stage IIA or lower disease, and 26.7% for stage IIB or greater disease. Median progression-free survival (PFS) was >487 days, and median time to treatment failure was 68.5 days. No difference in PFS by disease stage was observed. The safety profile of DD in CD25 low-expression disease was similar to that in CD25+ disease. These findings suggest that CD25 low expression does not preclude a meaningful clinical response to DD in patients with CTCL.

**Keywords:** Denileukin diftitox, antigens, CD25, mycosis fungoides, Sézary syndrome, cutaneous T-cell lymphoma

### Introduction

Cutaneous T-cell lymphomas (CTCLs) are characterized by the accumulation of malignant T lymphocytes in the skin [1,2]. The most common variants are mycosis fungoides (MF), featuring characteristic skin patches, plaques and tumors, and Sézary syndrome (SS), a leukemic variant featuring diffuse erythroderma with circulating malignant lymphocytes [1,2]. Approximately 50% of all cases of MF/SS express the interleukin-2 (IL-2) receptor, as demonstrated by immunohistochemical (IHC) staining, with high expression correlated with advanced-stage disease [3,4]. Depending on the presence of three subunits — alpha (CD25), beta (CD122), and gamma (CD13) — the affinity of the human IL-2 receptor is defined as low (CD25+ only), intermediate (CD122+/CD132+) or high (CD25+/CD122+/CD132+)

[4,5]. Both the intermediate- and high-affinity receptors cause internalization and signal transduction, whereas the low-affinity receptor does not [6].

Denileukin diftitox (DD; Ontak; Eisai Inc., Woodcliff Lake, NJ) is a genetically engineered fusion protein designed to target malignant or activated T cells based on their expression of the IL-2 receptor [5]. It combines the cytotoxic and membrane-translocating domains of the diphtheria toxin with human IL-2 [5,7]. Denileukin diftitox is approved in the USA for patients with relapsed CTCL that expresses CD25 on at least 20% of T cells in skin lesions analyzed by IHC (CD25+ disease) [8]. The availability of antibodies to the CD25 subunit, the expression of CD25 in higher quantities on cells than the CD122 subunit, and the inability of CD132 to be used as a selective marker (as it is a common subunit for many cytokine cell surface receptors) led to the use of CD25 screening as a tool for identifying patients who might respond to DD [9]. Based on IHC expression, patients with CD25+ skin lesions were assumed to express the high-affinity IL-2 receptor necessary for a response to DD, whereas patients with skin lesions exhibiting less than 20% CD25 expression (CD25 low-expression disease) were presumed to be unresponsive to DD [4]. This assumption may be incorrect for a number of reasons. First, the intermediate-affinity IL-2 receptor (CD122+/CD132+) can still cause internalization and signal transduction despite not expressing CD25 [6]. Second, the 20% cut-off for CD25 expression is arbitrary [5]. Third, the IHC assay is technically challenging and results can vary substantially [4,5,10]. Finally, CD25 expression can be variable at different tumor sites within the same patient [5,10].

Following an early case report of a response to DD in a patient with refractory CTCL (peripheral T cell lymphoma variant) and 50% CD25 positivity [11], a study of CD25 expression and response was published. In this study, patients with CD25 low expression (<20%) demonstrated a 20% response rate to DD compared with a 79% response rate in patients

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with CD25+ disease ( $p=0.01$ ) [4]. Moreover, there was marked discordance in CD25 expression results between the reference laboratory (using fresh, frozen biopsies) and a local laboratory (using paraffin-embedded fixed tissue) [4]. Questions remain as to whether DD should be restricted to patients with CD25+ disease. The insensitivity of the IHC assay to detect CD25 expression in skin biopsies suggests that it is an inadequate screening tool for excluding patients with CD25 low-expression lesions [4,10]. Moreover, *in vitro* studies have shown that, in the absence of the CD25 subunit, high levels of the other subunits can result in sufficient binding of DD to achieve cytotoxicity [6].

In a pivotal phase III trial (93-04-10) of two doses of DD (9 vs. 18  $\mu\text{g}/\text{kg}/\text{day}$ ), an objective response of 30% was reported in 71 patients with stage IB–IVA CTCL (MF Cooperative Group staging [12]) and CD25+ disease based on representative skin biopsies [5]. The recently reported, largest study of DD (L4389-11; NCT00050999) was a multicenter, randomized, double-blind, placebo-controlled phase III trial that evaluated the same doses of DD (9 or 18  $\mu\text{g}/\text{kg}/\text{day}$ ) versus placebo in 144 patients with stage IA–III MF/SS who had received up to three previous therapies [9]. The trial excluded patients with CD25 low-expression disease [9]. Using the same response criteria as the pivotal trial (93-04-10) [5,9], an overall response rate (ORR) was 44% for DD versus 16% for placebo [9]. Individuals with MF/SS who had been excluded from the placebo-controlled trial (L4389-11) because they had CD25 low-expression disease were enrolled in the study reported here (L4389-14), and all received treatment with DD.

## Materials and methods

### Patient eligibility

This multicenter, international, open-label trial (L4389-14; ClinicalTrials.gov Identification No.: NCT00051012) was conducted as a companion study to a previously published, placebo-controlled trial (L4389-11) [9] and included patients with a histologically confirmed diagnosis of MF or SS stages IA–III, according to the TNM (tumor, node, metastasis) and B staging classification of the MF Cooperative Group [12]. In the placebo-controlled trial, patients were randomly assigned to one of three arms: placebo, DD 9  $\mu\text{g}/\text{kg}/\text{day}$  or DD 18  $\mu\text{g}/\text{kg}/\text{day}$ , each administered on days 1 through 5 every 3 weeks for up to eight courses [9]. This companion trial (L4389-14) enrolled patients who were excluded from the placebo-controlled trial because they had CD25 low-expression disease (skin lesions exhibiting  $< 20\%$  CD25 expression) ( $n = 36$ ).

Key inclusion/exclusion criteria were carried over from the placebo-controlled trial, including age 18 years or older, stage IA–III MF/SS (staging system for MF based on the Bunn and Lamberg classification that did not include blood [12]; stage  $T_4B_2$  disease defined as SS [13]), receipt of three or fewer previous therapies, evaluable disease in skin or blood, life expectancy at least 12 months, and Eastern Cooperative Oncology Group performance status 0 or 1. Patients with nodal or visceral disease at baseline, but not significant blood involvement, were excluded.

This trial was approved by the institutional review boards of each participating medical center and was conducted

in compliance with the principles of the Declaration of Helsinki. All patients provided written informed consent before enrollment.

### Immunohistochemistry assay

Skin biopsies from representative skin lesions were obtained at baseline to determine CD25 expression in infiltrating lymphocytes. Blood samples were analyzed for the proportion of CD4+/CD3+/CD7– neoplastic lymphocytes. For CD25 low-expression disease, lesional skin biopsies had to demonstrate CD25 expression on  $< 20\%$  of malignant lymphocytes. CD25 expression was determined using IHC analysis of snap-frozen lesional specimens shipped on dry ice to a central laboratory (Quest Diagnostics, Inc., San Juan Capistrano, CA).

### Treatment

Patients received up to eight DD courses every 21 days for approximately 6 months. Each course was to comprise a 30 min intravenous infusion of DD 18  $\mu\text{g}/\text{kg}/\text{day}$  on days 1 through 5, with courses repeated every 21 days as tolerated. Patients received premedication with acetaminophen and an antihistamine 30–60 min before each infusion. These medications were continued during and after the dosing period, as clinically indicated. Pretreatment with corticosteroids was not permitted. Dose interruptions were permitted for certain toxicities. Patients who experienced a dose-limiting toxicity could have the dose of DD reduced to 9  $\mu\text{g}/\text{kg}/\text{day}$  at the investigator's discretion, provided that dosing safety criteria were met.

### Efficacy assessments

All patients were to be followed during treatment and after until the development of progressive disease (PD), relapse or initiation of a new anticancer therapy. Efficacy assessments were performed every 4 weeks for 12 months and every 3 months thereafter. Assessment of response was based on the percentage change in tumor burden at each study visit relative to baseline based on a global score of skin and, where applicable, lymph node and blood involvement. For patients with skin lesions involving more than 10% of the body surface area (BSA), all skin lesions were measured and multiplied by a weighting factor (1 for patch lesions, 2 for plaque lesions and 4 for tumors) to document disease extent in accordance with the severity-weighted assessment tool (mSWAT [14]). For patients with 10% or less BSA involvement, the sum of the areas of up to five representative skin lesions was determined and weighted for severity. Photography also was used to document the extent of skin disease. For patients with skin lesions and at least 20% abnormal peripheral lymphocytes at baseline, levels of circulating abnormal peripheral lymphocytes (CD3+/CD4+/CD7–), as calculated by fluorescence-activated cell-sorting analysis, were determined monthly from baseline. Computed tomography was performed at baseline to document visceral disease and baseline lymph node involvement. The percentage change from baseline in tumor burden was based on the average percentage changes in skin lesions weighted for severity and on levels of abnormal peripheral lymphocytes. Patients with lymph nodes at

baseline who developed biopsy-proven lymph node histology stage of LN3 or higher, indicating PD or relapse, had the bipерpendicular diameters of up to five representative nodes assessed by palpation or, in the case of an objective response, by computed tomography scan. The change in the sum of the bipерpendicular diameters from baseline was used to assess tumor burden. The average of the sum of the percentage change from baseline in skin score and, if relevant, the change in node and/or blood score was used to determine overall change in tumor burden and, hence, response.

### Primary endpoint

Response was determined at each disease assessment time point and compared with baseline. The primary efficacy endpoint was ORR, defined as the percentage of patients with complete response (CR; no clinical evidence of disease based on tumor burden assessments and photography corroboration with biopsy-confirmed absence of atypical cells by histopathology), clinical CR (CCR; no clinical evidence of disease based on tumor burden assessments and photography corroboration with either biopsy-confirmed presence of atypical cells by histopathology or no histopathology available) or partial response (PR;  $\geq 50\%$  reduction in measured tumor burden, but without meeting the criteria for CR or CCR). PD was defined as a 25% or greater increase in measured tumor burden compared with baseline, appearance of a new skin tumor lesion, appearance of newly involved lymph nodes (histology of LN3 or greater) or development of visceral disease. Stable disease was defined as an insufficient change in tumor burden to qualify for CR, CCR, PR or PD.

Each patient's response status at each study visit was to be reviewed by a Data Endpoint Review Committee (DERC) comprising CTCL experts who did not participate in the study. Responses (CR, CCR and PR) were considered documented if they were confirmed (same or better response) at three successive visit assessments over 6 or more weeks.

### Secondary endpoints

Secondary efficacy endpoints included progression-free survival (PFS), defined as the time from day 1 of treatment to first observation of tumor progression or death from any cause up to 30 days after the last dose of study drug, and time to treatment failure, defined as the time from day 1 of treatment to the date of PD or treatment discontinuation for toxicity.

### Supportive endpoints

Patients were assessed using the following disease symptom assessment tools to determine the clinical benefit of DD: time to onset of documented tumor response (time from day 1 of course 1 to first confirmed response), duration of documented tumor response (time from the date that a response was first documented until date of relapse, new cancer treatment or last observation), Physician's Erythroderma Severity Assessment, Physician's Global CTCL Severity Assessment, Patient's Pruritus Assessment, Patient's Global Skin Assessment, and quality-of-life data compiled from the FACT-G (Functional Assessment of Cancer Therapy – General) assessment tool.

### Safety assessment

Safety assessments included physical examinations (including vital signs), laboratory values and adverse events (AEs). In addition, patients were monitored at day 1 of each course before dose administration for the development of antibodies to DD. All AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA; version 6.1). The incidence of all AEs, drug-related AEs, and all AEs by severity were summarized according to MedDRA system organ class and preferred term. Treatment-emergent AEs were defined as all AEs that occurred after the first dose of study drug, or worsened or became related to treatment after the first dose. AEs were graded by severity according to a modified National Cancer Institute Common Toxicity Criteria (version 2) severity scale as mild, moderate, moderately severe or severe by each investigator. AEs were considered treatment related if they were reported by the investigator as either "possibly related," "probably related" or "yes, related" to DD. Adverse events were considered unrelated if they were reported as "unrelated," "probably not related" or "remote."

### Statistical analysis

The primary intent-to-treat efficacy analysis included all patients who received at least one dose of DD. Each patient's best-documented response was used in the analysis, and the 95% confidence interval (CI; two-sided, exact) of the ORR in each of the four patient groups was calculated. Secondary and supportive endpoints were analyzed using the Kaplan-Meier estimation method. The safety analysis population consisted of all treated patients. Safety analyses included determination of the frequencies of AEs, abnormal laboratory findings and abnormal vital signs.

### Role of the funding source

Ligand Pharmaceuticals contributed to the study design, provided study materials and funded the independent study monitoring. Ligand Pharmaceuticals and Eisai Inc. financially supported data analysis, and Eisai Inc. provided financial support for assistance with manuscript preparation (medical writing, editing and graphics).

## Results

### Patient disposition and demographics

This study, initiated in September 1995 and completed in October 2006, enrolled 36 patients with skin biopsies containing  $<20\%$  CD25 cells by IHC staining. Most patients were younger than 65 years, white and had early-stage ( $\leq$ IIA) disease at baseline (58.3%). At baseline, 15 patients (41.7%) had tumor stage disease IIB or tumors with erythroderma, nodes or visceral involvement (stages IIIA, IIIB, IVA or IVB); nine patients (25.0%) had a prior diagnosis of erythroderma, but none had a prior diagnosis of SS (Table 1).

### Efficacy

#### Primary endpoint

Patients received a median of four treatment courses (range: 1–8). Objective response rate, based on DERC assessment, was 30.6% (95% CI: 16.3, 48.1), with the majority of responses

Table I. Demographics and baseline characteristics.

Characteristic	Patients (n = 36)
Median age, years (range)	57.0 (26-80)
Age group, n (%)	
<65 years	27 (75.0)
≥65 years	9 (25.0)
Sex, n (%)	
Male	19 (52.8)
Female	17 (47.2)
Race, n (%)	
White	32 (88.9)
Asian	3 (8.3)
Black	1 (2.8)
Hispanic	0 (0.0)
Disease stage, n (%)	
Early (≤ IIA)	21 (58.3)
Late (≥ IIB)	15 (41.7)
Prior erythroderma diagnosis, n (%)	
Yes	9 (25.0)
No	25 (69.4)
Unknown	2 (5.6)
Prior SS diagnosis, n (%)	
Yes	0 (0.0)
No	34 (94.4)
Unknown	2 (5.6)
Prior MF/SS treatments, n (%)	
Phototherapy/photopheresis	23 (63.9)
Electron-beam radiation	18 (50.0)
Single chemotherapy	9 (25.0)
Combination chemotherapy	9 (25.0)
Interferon-α	9 (25.0)
Systemic retinoids	6 (16.7)
Topical chemotherapy	3 (8.3)
Cyclosporine A	1 (2.8)

MF, mycosis fungoides; SS, Sézary syndrome.

being partial responses (eight of 11 [73%] responding patients). Two patients had documented CCR and one patient had documented CR. Overall response rates were similar between patients with stage ≤ IIA disease (33.3%) and stage ≥ IIB (26.7%) disease at baseline (Table II).

### Secondary endpoints

Progression events were observed in approximately one-third (30.6%) of the 36 patients, with no notable differences by disease stage at baseline (Figure 1). Median

PFS was not reached at the time of the last data point but was more than 487 days [Table II; Figure 2(A)], although more than 50% of patients were progression-free at 200 days. Treatment failure events were observed for the majority (75%) of patients, with an estimated median time to treatment failure (time from day 1 of treatment to date of PD or treatment discontinuation for toxicity) of 68.5 days [Table II; Figure 2(B)]. A total of 12 (33.3%) patients discontinued treatment because of AEs.

### Supportive endpoints for clinical benefit

The Kaplan–Meier estimated median time to onset of response was more than 204 days (Table II). However, the observed median time to onset of response for responders was 43 days (range: 21–79 days). Relapse events were observed in five of the 11 (45.4%) patients who achieved an ORR. Responses for the remaining six patients were durable through the last observation at the close of the study. The estimated median duration of response was 340 days.

Clinically significant improvements in patients' pruritus and global skin self-report assessments were reported by approximately one-quarter and one-third of patients, respectively, during the treatment and post-treatment follow-up periods (Table III). A 20% or greater improvement in the patients' assessment of pruritus was reported by half of the patients, and 41.7% of patients reported 50% or greater improvement. A clinically significant improvement in the physician's assessment of erythroderma severity and global CTCL was reported for 13.9% and 27.8% of patients, respectively, during the treatment and post-treatment follow-up periods (Table III). A 20% or greater improvement in physician's assessment of global CTCL was reported in 52.8% of patients, and 38.9% of patients achieved 50% or greater improvement.

### Safety

Denileukin diftitox had an acceptable safety profile (Table IV). Thirty-five of the 36 (97.2%) patients experienced at least one AE, and 35 (97.2%) patients experienced a least one treatment-related AE. Twenty-three (63.9%) patients

Table II. Primary and secondary endpoints.

Endpoint	All patients (n = 36)	Stage ≤ IIA patients (n = 21)	Stage ≥ IIB patients (n = 15)
Primary endpoint, n (%)			
ORR	11 (30.6)	7 (33.3)	4 (26.7)
95% exact CI	(16.3, 48.1)	(14.6, 57.0)	(7.8, 55.1)
Best response			
CR or CCR	3 (8.3)	2 (9.5)	1 (6.7)
PR	8 (22.2)	5 (23.8)	3 (20.0)
SD	16 (44.4)	9 (42.9)	7 (46.7)
PD	9 (25.0)	5 (23.8)	4 (26.7)
Secondary endpoints,* median days (95% CI)			
PFS	>487 (NE)	>487 (NE)	129 (103, NE)
TTF	68.5 (46, 113)	72 (46, 204)	65 (36, 103)
TTR	>204 (NE)	>204 (NE)	>109 (NE)
DOR	340 (141, NE)	>400 (NE)	172 (58, 203)

CCR, clinical complete response; CI, confidence interval; CR, complete response; DOR, duration of response; NE, not estimable or not applicable; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; TTF, time to treatment failure; TTR, time to response.

\*Kaplan–Meier estimated median values.

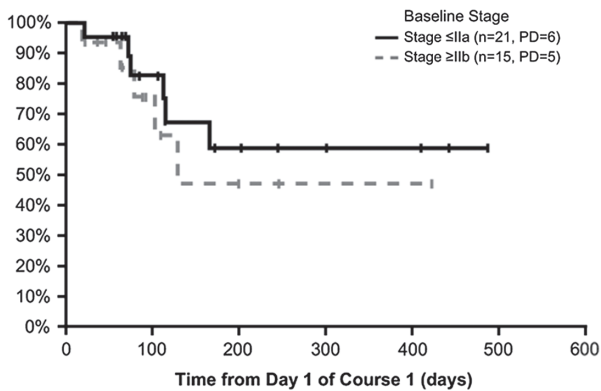


Figure 1. Kaplan-Meier plot of progression-free survival of patients with CD25 low-expression MF/SS in the intent-to-treat population ( $n = 36$ ), by baseline stage.

experienced at least one moderately severe or severe treatment-related AE. AEs with the highest overall incidence included pyrexia (55.6%), nausea (50.0%), fatigue (38.9%), rigors (36.1%), vomiting (36.1%) and peripheral edema (27.8%), and were all expected AEs associated with DD administration. The incidence of AEs declined notably after the initial 2–3 courses of DD treatment (Figure 3). Strategies to reduce adverse reactions to DD infusion in early courses (e.g. corticosteroid pretreatment) were not permitted.

In all, 23 of 36 (63.9%) patients experienced at least one serious AE (SAE), and 20 (55.6%) patients experienced at least one treatment-related SAE. Treatment-related moderately severe and severe SAEs with the highest overall incidence were nausea ( $n = 5$ ), vomiting ( $n = 2$ ), hypersensitivity ( $n = 2$ ), dehydration ( $n = 2$ ), dizziness ( $n = 2$ ), dyspnea ( $n = 2$ ) and capillary leak syndrome ( $n = 2$ ). Again, these were all expected AEs associated with DD administration. No incidents of deep vein thrombosis or pulmonary embolism were reported, and no patients died during the protocol-specified treatment period (up to and including 30 days post-treatment).

Denileukin diftitox did not appear to be myelosuppressive in this study, consistent with prior experience. Syndrome-related infections did not increase in frequency with continued exposure to DD (Figure 4).

## Discussion

Here we demonstrate that DD is effective (ORR of 30.6%) in patients with CD25 low-expression MF/SS (CD25 in <20% of

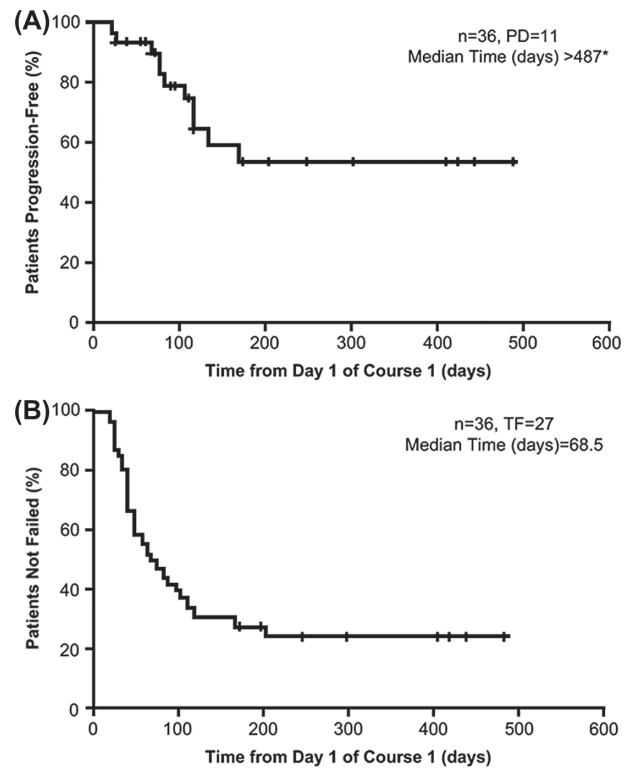


Figure 2. Kaplan-Meier estimates of (A) progression-free survival (PFS) and (B) time to treatment failure (TTF) for patients with CD25 low-expression disease ( $n = 36$ ). PFS was defined as the time from day 1 of course 1 to first observation of progressive disease (PD) or death from any cause. TTF was defined as the time from day 1 of course 1 to first observation of PD or study withdrawal for reasons other than tumor response or loss to follow-up. \*Final data point shown; median was not reached.

malignant T cells) determined by a standard IHC assay. Our findings and those of Talpur *et al.*, who reported a 20% ORR for patients with CD25 low-expression disease as measured by IHC of formalin-fixed, paraffin-embedded biopsy samples [4], confirm that DD is active in CD25 low-expression MF/SS.

The ORR of 30.6% in these patients with stage IA–III CTCL CD25 low-expression disease was similar to the 36% ORR observed in the pivotal phase III trial (93-04-10) of patients with stage IB–IVA CD25+ disease treated with DD 18  $\mu\text{g}/\text{kg}/\text{day}$ , although patients in the pivotal trial were heavily pretreated and response may be underestimated [5]. However, the response rate in the present analysis is lower than that reported in other studies in patients with CD25+ disease. In the placebo-controlled trial (L4389-11), an ORR of 49.1%

Table III. Summary of improvement in clinical benefit measurement scores.

Improvement, n (%)	Clinical disease assessment			
	Patient's clinical global skin	Patient's pruritus (VAS)	Physician's erythroderma severity	Physician's global CTCL (VAS)
Clinically significant during treatment	12 (33.3)	9 (25.0)	5 (13.9)	10 (27.8)
Clinically significant during post-treatment follow-up	12 (33.3)	10 (27.8)	5 (13.9)	10 (27.8)
$\geq 20\%$ improvement	NA	18 (50.0)	NA	19 (52.8)
$\geq 50\%$ improvement	NA	15 (41.7)	NA	14 (38.9)

CTCL, cutaneous T-cell lymphoma; NA, not applicable; VAS, visual analog scale.

Table IV. Overview of adverse events.

Event, n (%)	Patients (n = 36)
Any AE	35 (97.2)
Any serious AE	23 (63.9)
Discontinuation as a result of any AE	12 (33.3)
Treatment-related AE	35 (97.2)
Treatment-related serious AE	20 (55.6)
Discontinuation as a result of treatment-related AE	12 (33.3)
Moderately severe or severe treatment-related AE	23 (63.9)
Moderately severe treatment-related AEs ( $\geq 5\%$ incidence)	
Nausea	4 (11.1)
Vomiting	2 (5.6)
Asthenia	2 (5.6)
Peripheral edema	2 (5.6)
Pyrexia	2 (5.6)
Rigors	2 (5.6)
Hepatic enzyme increase	2 (5.6)
Rash	2 (5.6)
Chest discomfort	2 (5.6)
Lymphopenia	2 (5.6)
Dizziness	2 (5.6)
Syncope	2 (5.6)
Severe treatment-related AEs ( $\geq 2\%$ incidence)	
Hypersensitivity	2 (5.6)
Capillary leak syndrome	1 (2.8)
Dehydration	1 (2.8)
Headache	1 (2.8)
Tachycardia	1 (2.8)
Dyspnea	1 (2.8)
Diarrhea	1 (2.8)
Nausea	1 (2.8)
Hepatic enzyme increase	1 (2.8)
Pruritus	1 (2.8)

AE, adverse event.

was reported in patients with CD25+ disease who received DD 18  $\mu\text{g}/\text{kg}/\text{day}$  [9]. Of note, the placebo group in this trial had an ORR of 16%, and there was no difference between early- and advanced-stage disease [9,15]. In another study, response rates of 78.5% and 20% were reported for CD25+ patients and for patients with CD25 low-expression disease, respectively, who were receiving DD 18  $\mu\text{g}/\text{kg}/\text{day}$  [4]. Thus, not unexpectedly, response rates in our analysis of patients with CD25 low-expression disease appear to be lower than those reported in patients with CD25+ disease. These response data with DD are comparable to those observed with other available treatments, including the oral selective retinoid bexarotene, with a 45% response rate reported in patients with advanced CTCL who were refractory to at least one prior therapy [16]; the histone deacetylase inhibitor

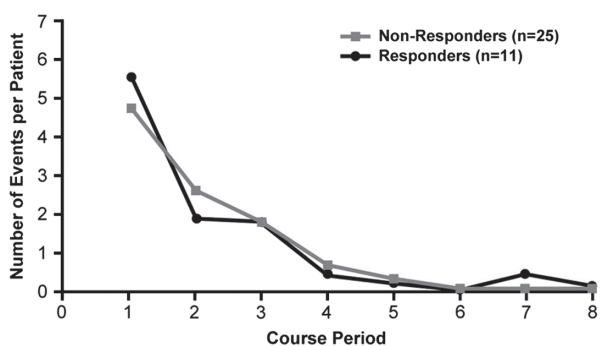


Figure 3. Treatment-related adverse events by course. During a course period (~3 weeks), an event was counted if it started on or after day 1 of that course. The number of events per patient was calculated by dividing the total number of events that occurred by the total number of patients exposed to study drug.

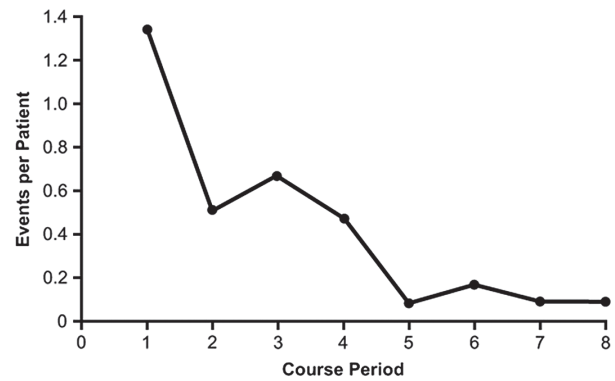


Figure 4. Syndrome-related infection by course. Any infection included bacterial, fungal, viral, fever and other infections. For fever, it must have occurred after the first 5 days of each course. During a course period (~3 weeks), an event was counted if it started on or after day 1 of that course. The number of events per patient was calculated by dividing the total number of events that occurred by the total number of patients exposed to study drug.

vorinostat, with a 30% response rate reported in patients refractory to two prior therapies [14]; and the histone deacetylase inhibitor romidepsin, with a 34% response rate reported in patients refractory to at least one prior therapy [17,18].

Median PFS was not reached at the time of the last data point and, therefore, was not estimable for either the CD25 low-expression group or the comparable DD group in the placebo-controlled trial (L4389-11). However, PFS at 200 days was approximately 50% in the CD25 low-expression group compared with approximately 70% in the placebo-controlled trial. While the outcome with CD25 low-expression patients was somewhat less favorable, a substantial proportion of these patients gained a meaningful clinical benefit, with an estimated median duration of response of 340 days (>11 months). In addition, both patient-reported and physician-reported benefits to patients were associated with documented responses to DD.

These outcomes suggest that screening patients by IHC for CD25 by a reference laboratory does not predict who will respond to DD. In this scenario, the IHC screening assay appears to be intrinsically insensitive or unreliable, suggesting that levels of CD25 expression might be misinterpreted in some patients. For example, in the pivotal phase III trial (93-04-10), 58% of all skin samples of patients with MF/SS screened for inclusion in the study were CD25+ [5]. However, results from multiple biopsies taken from different anatomic sites on different days from the same patients showed variable results, with 14 of 32 (44%) patients having conflicting results regarding CD25 expression [5]. Another study found a poor correlation between the reference laboratory and a local laboratory when patients' biopsies were divided into two, with one half sent to each laboratory and assessed for CD25 expression [4].

Whether this variability is biologic or secondary to the inherent insensitivity of the screening assay is unclear. A study assessing the immunophenotype of T-cell tumors simultaneously sampled at different sites found that CD25 expression was highly variable, suggesting that biology accounts for at least some of this variability [10]. CD25 was expressed most intensely by lymphoma cells in the epidermis and peripheral blood. Moderate CD25 intensity was found in tumor cells within the dermis and lymph node sinusoids. The lowest

levels of CD25 intensity were found in tumor cells in the lymph node parenchyma. Moreover, when IL-2 antibody titers were assessed (as described elsewhere [5]), CD25 low expression did not affect anti-IL-2 antibody titers (data not shown). Similarly, CD25 receptor status did not influence the anti-denileukin diftitox antibody.

In the open-label study reported here (L4389-14), the highest tested dose (18 µg/kg/day) of DD from the initial pivotal trial (93-04-10) was used because, in earlier studies, this dose showed a numerically higher documented response rate (36%) with tolerable AEs, compared with the response rate with 9 µg/kg/day (23%) [5]. However, this difference did not reach significance, possibly owing to the small sample size [5]. In the present study (L4389-14), DD 18 µg/kg/day had an acceptable safety profile in patients with CD25 low-expression disease, consistent with other studies of DD. Adverse events were generally mild to moderate in severity, were reversible, and declined notably after the initial 2–3 courses of DD. Moderately severe or severe capillary leak syndrome occurred in two of 36 (5.6%) patients, but was monitored and managed with limited or no interruption of DD treatment. Denileukin diftitox did not appear to be immunosuppressive. The incidence of all AEs, including infections, did not increase in frequency with continued DD exposure.

The results from this study demonstrate that documented responses with DD are achievable and durable for patients with MF/SS who express CD25 on less than 20% of tumor tissue T cells, and highlight the need for better biomarkers that can predict the response to DD.

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