Chimeric Fusion Protein Toxin DAB\textsubscript{486}IL-2 in Advanced Mycosis Fungoides and the Sezary Syndrome: Correlation of Activity and Interleukin-2 Receptor Expression in a Phase II Study

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DAB\textsubscript{486}IL-2 is a recombinant toxin with the cell surface-binding domain of diphtheria toxin (DT) replaced by interleukin-2 (IL-2). To correlate clinical response with expression of components of the IL-2 receptor (IL-2R), 14 patients with cutaneous T-cell lymphoma (CTCL) received five daily 90-minute infusions every 21 days. There were no complete responses, 1 partial response (PR), 2 major biologic effects (major cutaneous improvement without change in circulating neoplastic cells), 3 stable disease (SD), and 8 progressive disease (PD). Responders had easily detectable expression of CD25 (Tac; α-chain of IL-2R) in skin, and in two responders expression of the β chain of the IL-2 receptor (β-IL-2R) was detectable by reverse transcriptase-polymerase chain reaction. CD25 was also detected in 8 of 11 SD or PD patients, with β-IL-2R in 3 of 8 SD or PD patients. Two of the three respondents had anti-DT antibodies before treatment. Reversible increased hepatic transaminases occurred in 13 of 14 patients during the first course, with decreased frequency in repeated courses. The maximal serum concentration after the first infusion of DAB\textsubscript{486}IL-2 varied (1,369 ± 1,155 ng/mL [mean ± SD]; n = 14; range, 55 to 3,999 ng/mL) with a short half-life (T\textsubscript{1/2}β = 0.21 ± 0.12 h [mean ± SD]; range, 0.099 to 0.57 h). The area under the concentration curve varied inversely with anti-DT antibody titer. We conclude that DAB\textsubscript{486}IL-2 has valuable activity in certain patients with CTCL. Expression of the IL-2R may be necessary but is not sufficient to predict response. This is a US government work. There are no restrictions on its use.

CUTANEOUS T-cell lymphoma (CTCL) is a generally indolent neoplasm (usually of CD4	extsuperscript{+} T-helper cells) that may manifest as mycosis fungoides (MF), with cutaneous patches, plaques, and tumors, or as the Sezary syndrome (SS), referring to erythroderma accompanied by circulating neoplastic lymphocytes. A clinicopathologic staging system for CTCL has been described that takes into account the presence of skin, lymph node, and visceral disease. Whereas durable complete remissions have been reported in patients with early stage disease using a number of modalities, including topical nitrogen mustard, total skin electron beam irradiation, 8-methoxypsoralen plus UVA light (PUVA) with or without α-interferon, and α-interferon alone, the treatment and successful palliation of more advanced disease has proven more difficult. Response durations to cytotoxic chemotherapies in advanced disease have been short and toxicities have been significant. The failure of conventional cytotoxic chemotherapies in these and other indolent, slowly proliferating lymphoid neoplasms has prompted the development of alternative biologically targeted or immunomodulatory therapies, including interferon, photopheresis, and thymopentin.

DAB\textsubscript{486}IL-2 is a recombinant chimeric fusion protein derived from a gene in which the ADP-ribosylating and membrane translocating portions of diphtheria toxin (DT) have been fused by recombinant techniques in the same reading frame with a synthetic interleukin-2 (IL-2) gene. The protein selectively binds and kills cells bearing the high-affinity IL-2R complex. Phase I studies of the agent in patients with refractory hematopoietic malignancies showed that DAB\textsubscript{486}IL-2 was well tolerated and exhibited antitumor activity. Of patients with MF or SS who were treated in various phase I studies at different doses and schedules, approximately 20% were reported to have responded, including 1 patient who had a complete response of more than 36 months' duration.

This study was designed to assess the efficacy of DAB\textsubscript{486}IL-2 in patients with advanced MF or SS when administered according to a fixed dose and schedule, and to correlate response with the expression of the IL-2R on tumor cells. We found that DAB\textsubscript{486}IL-2 has clinically useful activity in some patients with MF or SS. Although expression of at least some component of the high-affinity IL-2R is detectable in responding patients, IL-2R expression alone does not appear sufficient to predict response to treatment with this regimen.

PATIENTS AND METHODS

Patients. Fourteen patients with histologically proven MF or SS stages IIB to IVa with or without prior therapy or stages I-IIA refractory to prior therapy, and Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 were entered into the study. Patients were excluded if they had New York Heart Association class III or IV cardiac disease, impaired hepatic or renal function, central nervous system (CNS) involvement, or evidence of hepatitis B or human immunodeficiency syndrome (HIV) infection. Informed consent to the experimental nature of the therapy was obtained from all patients according to National Cancer Institute (NCI) Institutional Review Board guidelines. Therapy directed at the patient’s CTCL was discontinued 30 days before entry on study, unless obvious progression of disease occurred, and then DAB\textsubscript{486}IL-2 therapy continued.

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nenced less than 30 days after discontinuation of prior therapy. Although there was no requirement for prior demonstration of IL-2R on the patient’s tumor cells before entry onto this study, all patients underwent initial and follow-up biopsies for characterization of IL-2R receptor expression. Accrual to the study ceased after 14 patients because of the availability for use in clinical trials of DAB4861L-2, a second-generation IL-2R-targeted fusion protein toxin with more favorable pharmacokinetic features.

**DAB4861L-2.** DAB4861L-2 is a recombinant fusion toxin produced by Escherichia coli and isolated by immunoaffinity chromatography (using polyclonal horse anti-DT Ig) and high performance liquid chromatography.15 The purified, formulated protein was supplied by Seragen, Inc (Hopkinton, MA).

**Regimen.** A course of treatment consisted of DAB4861L-2 at 0.2 mg/kg administered intravenously over 90 minutes daily for 5-days, followed by observation for 16 days. This dose and schedule was based on phase I studies that characterized the maximal tolerated dose as that which caused reversible, noncumulative, up to 10-fold elevation of hepatic transaminases. This dose was below the level at which unacceptable toxicity characterized by reversible hemolytic anemia, thrombocytopenia, and renal insufficiency occurred. Toxicity was scored according to the NCI common toxicity criteria.20 Patients were treated for four courses in the absence of grade 4 toxicity. Patients with grade 3 toxicity that returned to baseline were retreated at a 75% dose, and those with persistent grade 1 or 2 toxicity received a 50% dose. The study agent was discontinued in patients with grade 4 or persistent grade 3 toxicity. Concomitant therapies directed against the primary disease, including topical steroids as that which caused reversible, noncumulative, up to 10-fold toxicity received a 50% dose. The study agent was discontinued in patients with grade 4 or persistent grade 3 toxicity. Concomitant therapies directed against the primary disease, including topical steroids as that which caused reversible, noncumulative, up to 10-fold elevation of hepatic transaminases. This dose was below the level at which unacceptable toxicity characterized by reversible hemolytic anemia, thrombocytopenia, and renal insufficiency occurred. Toxicity was scored according to the NCI common toxicity criteria.20 Patients were treated for four courses in the absence of grade 4 toxicity. Patients with grade 3 toxicity that returned to baseline were retreated at a 75% dose, and those with persistent grade 1 or 2 toxicity received a 50% dose. The study agent was discontinued in patients with grade 4 or persistent grade 3 toxicity. Concomitant therapies directed against the primary disease, including topical steroids, topical nitrogen mustard, electron beam therapy, PUVa, or chemotherapy, were not allowed during the study period. Emollients were allowed ad libitum and antihistamines were administered as clinically indicated.

Response assessment was based on the measurement of clinically evaluable disease in skin, lymph nodes, blood, and bone marrow. Response in skin was determined by change in the size of target lesions (5 per patient) and the percentage of total skin surface involved by disease as assessed by two independent observers and documented with skin photographs. Response in the blood was determined by comparing the absolute numbers and percentage of circulating atypical lymphocytes seen on peripheral smear and by immunophenotypic analysis of peripheral blood mononuclear cells at the onset of therapy and after each cycle of treatment. Lymph node response was determined by measurement of palpable nodes and by CAT scans.

A complete response (CR) was defined as the complete resolution of all clinically evident disease confirmed pathologically by biopsy and lasting at least 4 weeks. A partial response (PR) required at least a 50% reduction in the sum of all measurable and assessable disease that lasted at least 4 weeks. A biologic effect (BE) was defined as a less than 50% reduction in measurable disease that lasted at least 4 weeks. Progression of disease (PD) was defined as a 25% or greater increase in circulating neoplastic cells, appearance of new visceral lesions, or new skin lesions in two or more sites. Response duration and survival were measured from the first day of treatment.

**Immunohistochemical studies.** Skin biopsies were obtained from all patients for routine histopathology and immunohistochemical studies before and after completion of therapy. Paraffin sections were stained for UCHL1, L252, L-26, and KP1. Frozen sections were stained for CD3, CD2, CD5, T-cell receptor (TCR) aδ, TCRγδ, HLA-DR, CD4, CD8, CD7, CD25, CD56, CD16, CD19, CD11b, and CD57. Immunophenotyping of peripheral blood mononuclear cells was performed before the onset of the study, on day 1 before drug administration, and weekly thereafter.

**Other assays.** Soluble IL-2R (sIL-2R) was measured by enzyme-linked immunosorbent assay (ELISA) using previously described methods before and at the completion of therapy.16 Serum samples were assayed by ELISA for antibodies to DT, DAB4861L-2, IL-2, E coli, and horse IgG.17 Serial blood samples for pharmacokinetic analysis were obtained on days 1 and 5 of the first and final cycles of therapy. Serum DAB4861L-2 concentrations were measured using a previously described bioassay.23

**Molecular analysis of IL-2R.** Skin biopsies and peripheral blood mononuclear cells were assessed for IL-2R gene expression. Skin biopsies from involved areas were obtained and snap-frozen in liquid nitrogen. Peripheral blood mononuclear cells were prepared by ficoll–hypaque separation from whole blood. Total RNA was extracted from skin and peripheral blood mononuclear cells using RNASol, as described elsewhere.22 cDNA was synthesized using Moloney murine reverse transcriptase (Bethesda Research Labs, Gaithersburg, MD). Polymerase chain reaction (PCR) amplification of synthesized cDNA was performed as previously described.22 The oligonucleotides used for amplification of the p55 and p75 moieties of the IL-2R were, for the p55 (Tac; or IL-2R α-chain), bp 241-256 and 812-828 of the mRNA nucleotide sequence, giving a 587-bp fragment; and for the p75 (or IL-2-R β-chain), bp 579-596 and 1018-1034, giving a 455-bp fragment.23 The PCR cycle for amplification was 94°C for 1 minute, 50°C for 2 minutes, and 72°C for 2 minutes for 35 cycles. PCR products were separated on 2% agarose gels and blotted onto nitrocellulose filters. The blots were hybridized in 6× SSC (0.9 mol/L NaCl, 0.09 mol/L Na citrate) at 50°C to radiolabeled oligonucleotides homologous to internal sequences within each amplified gene (IL-2R p55; bp 366-380; IL-2R p75, bp 614-630). The blots were washed at high stringency and were exposed on XAR film (Eastman Kodak, Rochester, NY).

**RESULTS**

**Patient characteristics.** Fourteen patients were accrued (7 female and 7 male), with a median age of 58 years. Clinical features of the patients, with extent of disease estimated according to standard staging definitions, are listed in Table 1.25 Five patients had patches and plaques, 5 had tumors, and 4 had erythroderma. Five patients had circulating neoplastic lymphocytes, 3 had effaced lymph nodes, and 2 had bone marrow involvement (stage IIB). Twelve of 14 had received prior cytotoxic chemotherapy, but 2 had received only topical therapies, including topical nitrogen mustard and total skin electron beam irradiation. One patient (no. 6) had received therapy with DAB4861L-2 as part of a phase 1 study conducted at another institution and had demonstrated response.

**Response and survival.** One of 14 patients achieved a clear PR (Table 2). This patient (no. 3) had failed to respond to multiple prior therapies and had generalized patches and plaques without adenopathy or visceral involvement. After two courses of therapy, all evaluable skin lesions flattened and became less erythematous, and pruritus resolved completely. Improvement was maintained through six courses, when therapy was stopped because of limited DAB4861L-2 availability. Figure 1A and C shows the resolution of one of the patient’s skin lesions after four courses of treatment. Repeat skin biopsies in initially involved areas demonstrated dramatic reduction of the atypical lymphocytic infiltrate (as shown by comparing Fig 1B and D). Staining with anti-Tac...
also became negative. The patient’s response fell short of a CR only because one skin biopsy from a previously clinically involved area continued to demonstrate residual atypical lymphocytic infiltrate despite clinical clearing of all other involved areas. The response was maintained for 32 weeks without other topical or systemic therapies.

Patients no. 6 and 7 each had a clear-cut BE of treatment with DAB4IL-2, defined as a response in a disease parameter that was objective but fell short of the required greater than 50% overall improvement for a PR. Both patients had erythroderma and circulating Sezary cells, and both had positive CD25 staining of their skin infiltrates; the circulating Sezary cells in patient no. 6 were positive for CD25, whereas those in patient no. 7 lacked CD25 expression. Figure 2A shows an on-study skin photograph of patient no. 6, demonstrating erythroderma and multiple small plaques, which improved after four courses (Fig 2B); however, the patient had no change in the number of circulating Sezary cells. This patient had received prior therapy at a lower dose with DAB4IL-2 on a phase I study, and a similar result had been noted, with improvement in the skin without change in the number of circulating neoplastic lymphocytes.19 Similarly, patient no. 7 had marked and clinically beneficial improvement of erythroderma, dermal edema, exfoliation, and pruritis (compare Fig 2C with D). Both patients no. 6 and 7 fell short of a PR because in neither case was there a decrease in circulating Sezary cells after four courses of treatment, as shown in Fig 3B. Nonetheless, clinically there was marked improvement in cutaneous disease (Fig 2). Three patients demonstrated stable disease after receiving four courses of therapy. Eight patients had therapy discontinued because of disease progression (3 after 1 course, 2 after 2 courses, and 1 after 3 courses). Two lots of DAB4IL-2 were used during the study, each with similar potency and purity. Responding patients no. 6 and 7 received treatment with both lots, whereas responding patient no. 3 received treatment with only one of the lots. Nonresponding patients also received treatments using both lots.

Seven of 14 patients are alive at a median follow-up of 21 months (range, 13 to 27). Six patients died from progressive disease after receiving salvage chemotherapies. Patient no. 9 experienced sudden death at home 3 weeks after the completion of four courses of therapy and was found at autopsy.

### Table 1. Patient Characteristics

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<th>Patient No.</th>
<th>Stage*</th>
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Abbreviations: T1, plaques <10% skin surface; T2, plaques >10% skin surface; T3, skin tumors; T4, erythroderma; EBRT, total skin electron beam irradiation; CHEMO, cytotoxic chemotherapy; IFN, interferon-α; HN2, topical nitrogen mustard; DAB-IL2, DABIL-2 toxin; ND, not determined.

* Staging according to Sausville et al.20
† LN classification according to Sausville et al.20

### Table 2. CR and IL-2R Expression

<table>
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<th>Patient No.</th>
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Abbreviation: ND, not determined.

* Tac expression in the skin determined by immunohistochemical staining using anti-CD25 antibody: 4+, >75% tumor cells (+); 3+, 50% to 75% tumor cells (+); 2+, 25% to 50% tumor cells (+); 1+, 5% to 25% tumor cells (+); - , no or rare cells (+).

† IL-2Rβ expression determined by hybridization of electrophoresed and blotted products of RT-PCR with IL-2Rβ probe, as described in Patients and Methods.

‡ Peripheral blood Tac determined both by immunophenotypic analysis using anti-CD25 and by RT-PCR as described in Patients and Methods.
Fig 1. PR of patient no. 3. (A) Pretreatment skin lesion representative of multiple small plaques diffusely covering the body surface. (B) Pretreatment hematoxylin and eosin skin biopsy, again demonstrating epidermotropism with marked exocytosis. (C) Flattening and healing of lesions after four courses of DAB4IL-2. (D) Posttreatment biopsy showing substantial but incomplete clearing of the infiltrating cells.

to have coronary artery disease and an acute myocardial infarction. Patient no. 5 died of progressive visceral lymphoma.

IL-2R expression. Although not a prerequisite to study entry, attempts were made to determine the IL-2R expression by patients' tumor cells in the skin and node, and on circulating Sezary cells by immunohistochemistry and flow cytometry using the anti-Tac antibody (which detects the p55 α-chain of the IL-2R).26,27 As shown in Table 2, 11 of 14 patients had evidence of anti-Tac staining in skin infiltrates (1+ in 5, 2+ in 2, and 4+ in 4), including the three responders (patients no. 3, 6, and 7). Four of five patients with Sezary syndrome had anti-Tac staining on the malignant lymphocytes, and 1 of these (patient no. 6) had a BE. The other Sezary patient who obtained a BE after treatment with DAB4IL-2 (patient no. 7) had no anti-Tac staining on the circulating neoplastic lymphocytes, although skin infiltrates were Tac(+). The immunophenotype on this patient's circulating tumor cells was CD3+, CD4+, CD8+, CD57+, CD7+, TCRαβ+, an unusual phenotype for Sezary cells.

Because a sensitive antibody with low background staining was unavailable for immunostaining for the p75 (β-chain of the IL-2R) was not available at the time of this study, expression of the p75 component of the IL-2R in involved skin and blood was determined by PCR amplification of reverse transcribed mRNA (RT-PCR). As shown in Table 2, p75 expression was demonstrated in involved skin in 5 of 11 patients tested. All 5 patients expressing p75 were also Tac(+), whereas 4 were Tac(+), p75(−), and 2 lacked expression of both Tac and p75. Of the 3 responding patients, all demonstrated cutaneous expression of at least one component of the IL-2R, 2 with both Tac and p75, and 1 (patient no. 7) with Tac alone. However, not all patients whose tumors expressed either Tac or Tac plus p75 responded: 3 of the 5 patients expressing both Tac and p75 were nonresponders. Two (patients no. 4 and 8) of these had cutaneous tumors, and 1 (no. 2) manifested readily apparent increase of CD25 expression during treatment (see below). Nonresponders also included 3 of the 4 patients who demonstrated Tac without p75.

Effects of DAB4IL-2 on normal lymphocytes and Sezary cells. Immunophenotypic analysis of peripheral blood mononuclear cells before treatment and on days 5 through 8 of the first or second courses (Fig 3) showed significant differences in the total number of T cells, measured as CD3+ cells, only in patient no. 14, who experienced a marked decrease in T cells after one course of therapy (Fig 3A). There were minor decreases in the normal T-cell population
Fig 2. Response of patients no. 6 and 7. (A) Pretreatment view of patient no. 6 with erythroderma and excoriated plaques. (B) Patient no. 6, after four courses, with diminution of extent of active plaque lesions. (C) Pretreatment view of patient no. 7, with marked exfoliation, erythroderma, and pedal edema. (D) Patient no. 7, after four courses of DAB-IL-2.

Fig 3. Effect of DAB-IL-2 on normal and malignant T cells. (A) Normal T cells. The baseline number of normal T cells present in the peripheral circulation of patients without evidence of blood infiltration by CTCL was quantitated within 2 weeks of initiation of treatment (Pre-Rx), with posttreatment measurements (Post-Rx) obtained on day 4 (no. 9, △), day 5 (no. 5, □); day 6 (no. 10, ▲; no. 12, ○; no. 14, ●) and day 8 (no. 3, ▽; no. 4, ▼) of course 1 (—). In patients no. 1 and 8, Pre-Rx represents specimens obtained on course 2 (— - -), day 1 and Post-Rx represents specimens obtained on course 2, day 8 (no. 1, ●) and course 2, day 9 (no. 8, ▐). (B) Sezary T cells. Patients with abnormal circulating CTCL cells (in all cases morphologically abnormal) had quantitation of the number of circulating tumor cells in all cases in course 1, day 1 (Pre-Rx). Posttreatment (Post-Rx) quantitation of circulating tumor cells occurred on course 1, day 5 (no. 13, ■), day 6 (no. 11, □), day 8 (no. 2, ●; no. 6, ▼), and day 9 (no. 7, ▼).
of patients no. 1, 4, 5, 8, 9, 10, and 12. Circulating neoplastic cells in patients no. 2 and 7 increased somewhat after the initial treatment, despite the fact that patient no. 7 had marked clinical improvement in the skin (Fig 2). Patients no. 6 and 11 had slight decreases, and patient no. 13 had essentially no change in circulating tumor cell burden.

A striking finding emerged in the case of patient no. 2, who was distinctive in that, at baseline, the patient had two populations of tumor cells based on differential expression of CD3 detected by flow cytometry, a feature that may be of diagnostic utility for blood involvement by CTCL. Fig 4 demonstrates that during three courses of treatment with DAB4L-2, the CD25 expression in the subpopulation of T cells that expressed a more abundant level of CD3 actually increased, a trend that was apparent even after only one course of treatment. This result suggests that treatment with DAB4L-2 may have selected for emergence of a subclone that expressed more abundant levels of the IL-2R, the molecular target for DAB4L-2. Alternatively, this finding may simply reflect evolution of the disease in this patient.

Toxicity. The toxicities noted in this study were primarily those anticipated from the prior phase I DAB4L-2 studies. Elevation of hepatic transaminases occurred in 75% of courses administered, and occurred in 13 of 14 patients (9 grade 1 or 2 and 4 grade 3) after the first course of treatment, but the incidence and magnitude of this change deceased with subsequent courses and occurred in only 3 of 6 patients (all grade 1 or 2) completing four courses of treatment.

Constitutional symptoms, including chills and fever managed by acetaminophen and diphenhydramine, occurred in 83% of courses, with grade 1 or 2 hypotension in 28% of courses administered, and nausea or vomiting in approximately 20% of courses. One patient (no. 5) developed reversible bronchospasm without hypotension that resolved with administration of intravenous steroids. Hypoalbuminemia occurred to a generally minor extent in 58% of courses with mild transient proteinuria in 4 patients, and edema to a minor extent in approximately 20% of cases. Reversible minor increases in creatinine related to the administration of DAB4L-2 occurred in 2 patients, and in 1 of these azotemia was accompanied by evidence of hemolysis and thrombocytopenia indicative of a mild hemolytic-uremic syndrome, as has been described in prior phase I studies.17,18 This abnormality reversed without specific treatment and did not recur when the patient was retreated with a lower dose. Myelosuppression was not observed.

Staphylococcal sepsis complicated 15% of courses administered. Although all patients were initially treated with central venous catheters, no patient was neutropenic at the time of the septic episodes, and these were attributed to line contamination in patients with impaired skin integrity. Attempts were then made to treat patients using peripheral access wherever possible.

Serum antibodies and sIL-2R. There was a variable level of neutralizing anti-DT antibodies pretherapy, with 6 patients demonstrating no neutralizing antibodies, as shown in Table 3. Patient no. 6, who showed the highest antibody titers, had received DAB4L-2 on a prior study. Patient no. 5, who also showed a high pretreatment titer of anti-DT antibody,
very rapid clearance mechanism in patients with high anti-DAB-IL-2

other trials with DAB48JL-2,L7s'8 the clearance was rapid, 0.57 h (0.21

anti-DAB486iL-2 antibody levels. This result could arise DAB4861L-2 was not detected. These patients had the highest

Patient

Table 4. Initial Pharmacokinetics of DAB48IL-2

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>T1/2α (h)</th>
<th>C1 (h)</th>
<th>Vf (L/kg)</th>
<th>AUC (mg·h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,432</td>
<td>0.47</td>
<td>0.13</td>
<td>7.5</td>
<td>0.08</td>
<td>1.740</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2,606</td>
<td>0.10</td>
<td>0.30</td>
<td>4.4</td>
<td>0.01</td>
<td>2.66</td>
</tr>
<tr>
<td>4</td>
<td>3,999</td>
<td>0.10</td>
<td>0.24</td>
<td>4.0</td>
<td>0.007</td>
<td>4.42</td>
</tr>
<tr>
<td>5</td>
<td>678</td>
<td>0.32</td>
<td>0.20</td>
<td>20.2</td>
<td>0.17</td>
<td>0.53</td>
</tr>
<tr>
<td>6</td>
<td>3,363</td>
<td>0.09</td>
<td>0.57</td>
<td>8.3</td>
<td>0.01</td>
<td>1.97</td>
</tr>
<tr>
<td>7</td>
<td>1,003</td>
<td>0.11</td>
<td>0.23</td>
<td>15.2</td>
<td>0.03</td>
<td>1.15</td>
</tr>
<tr>
<td>8</td>
<td>1,198</td>
<td>0.12</td>
<td>0.19</td>
<td>16.2</td>
<td>0.05</td>
<td>0.69</td>
</tr>
<tr>
<td>9</td>
<td>1,318</td>
<td>0.30</td>
<td>0.10</td>
<td>7.8</td>
<td>0.06</td>
<td>1.51</td>
</tr>
<tr>
<td>10</td>
<td>719</td>
<td>0.09</td>
<td>0.57</td>
<td>16.6</td>
<td>0.07</td>
<td>1.03</td>
</tr>
<tr>
<td>11</td>
<td>947</td>
<td>0.23</td>
<td>0.15</td>
<td>16.6</td>
<td>0.07</td>
<td>1.03</td>
</tr>
<tr>
<td>12</td>
<td>268</td>
<td>0.15</td>
<td>0.23</td>
<td>86.3</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>13</td>
<td>399</td>
<td>0.06</td>
<td>0.16</td>
<td>12.0</td>
<td>0.02</td>
<td>0.85</td>
</tr>
<tr>
<td>14</td>
<td>596</td>
<td>0.19</td>
<td>0.19</td>
<td>13.5</td>
<td>0.05</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Course 1, day 1 pharmacokinetic parameters were calculated following bioassay of DAB48IL-2 as described in Patients and Methods. Patient no. 2 had levels below detection throughout the period of sampling after the infusion.

experienced a clinical acute hypersensitivity reaction on day 5. Four patients had detectable anti—IL-2 antibodies before therapy. There was no clear relationship between clinical response and pretreatment antibody titers for anti-DT, anti-DAB48IL-2, or anti-IL-2. Antihorse IgG titers were elevated after therapy only in the patient who had received prior DAB48IL-2.

All patients had elevated sIL-2R levels before therapy (range, 680 to 11,470 U/mL; normal, <500 U/mL), as shown in Table 3. There was no correlation between level of sIL-2R and clinical response, consistent with prior demonstration in vitro that the presence of sIL-2R at concentrations up to 50,000 U/mL does not block the cytotoxic effect of DAB48IL-2.

Two of the three responders and one nonresponder demonstrated a decrease in sIL-2R after therapy.

Pharmacokinetics. Pharmacokinetic parameters were determined by noncompartmental analysis on the first and last doses of the initial and fourth course of therapy. Table 4 summarizes the parameters after the first dose of the first course. Marked interpatient variability after the infusion was noted: maximal serum concentrations (Cmax) achieved during the initial infusion ranged between 55 and 3,999 ng/mL, and the area under the concentration curve (AUC) between 0.5 mg·h/L and 4.4 mg·h/L. Likewise, there was substantial intrapatient variability between doses. For example, patient no. 12 had a Cmax of 719 ng/mL after the first dose and 175 ng/mL after the fourth course of treatment. As described in other trials with DAB48IL-2, the clearance was rapid, with a range of 4.4 L/h to 86 L/h (17.4 ± 21.2 L/h [mean ± SD]) and a terminal half-life (T1/2α) between 0.09 and 0.57 h (0.2 ± 0.1 [mean ± SD]). No clear correlation could be made between the calculated pharmacokinetic parameters and the presence of Sezary cells. After 8 of 39 courses, DAB48IL-2 was not detected. These patients had the highest anti-DAB48IL-2 antibody levels. This result could arise from interference of the antibody with the bioassay or of a very rapid clearance mechanism in patients with high anti-

DAB48IL-2 levels. There was moderate correlation between the existence or formation of anti—DAB48IL-2 antibodies and a reduced AUC (r = −.46, Spearman’s correlation; Fig 5). However, rapid clearance was apparent even in patients such as patient no. 13, who showed no evidence of preexistent or intercurrent antibodies to DAB48IL-2 (Table 4). This finding underscores the likely involvement of mechanisms other than antibody-mediated clearance to account for rapid disappearance of DAB48IL-2.

DISCUSSION

Our results using DAB48IL-2 in this phase II study in a group of advanced-stage patients with CTCL are notable for objective and clinically valuable responses in 3 of 14 extensively pretreated patients, all of whom showed evidence of at least some component of the IL-2R on their cutaneous tumor cells by immunostaining. Demonstration of clinical activity of DAB48IL-2 in MF or SS has been previously reported in phase I studies by LeMaistre et al,I7*I8 who reported 2 responses (1 PR, 1 minor response) in 5 CTCL patients, and Hesketh et al,19 who noted 1 CR and 2 PR in 5 treated patients. The current study by its design precluded the concomitant use of topical agents, including corticosteroids, in contrast to the previous studies,1718 in which patients could continue use of topical corticosteroids after entry onto the study. The responses reported here therefore unequivocally represent the clinical activity of DAB48IL-2 acting as a single agent.

The IL-2R is expressed in a high-affinity form on normal T lymphocytes after activation by antigen or lectin and is constitutively expressed by HTLV-1–infected cells. However, components of the IL-2R are variably expressed by non-Hodgkin’s lymphomas, Hodgkin’s disease, hairy cell
leukemia, and nonlymphocytic leukemia cells. Whereas the receptor exists in several forms depending on which of its component proteins (p55: α-chain, Tac; p75: β-chain; p64, γ-chain) are present, only cells bearing the high-affinity or heterotrimeric (αβγ) form of the receptor are capable of efficiently binding and internalizing the ligand and, likewise, the fusion toxin. Detection of the high-affinity isoform on clinical specimens has been limited by the lack of useful antibodies to the p75 and p64 components of the receptor for immunohistochemical studies and has relied on available antibodies directed against the Tac or p55 component.

The expression of Tac in the skin and on circulating atypical lymphocytes in patients with MF and SS has been variable in previously published series. Whereas Tac expression had initially been described as low or negative on the circulating neoplastic cells of patients with Sezary syndrome, its expression by immunohistochemical staining in the skin of patients with MF and SS has been reported in up to 75% of cases. Because both histopathologic and immunohistochemical studies show that the dermal and epidermal infiltrates in patients with MF or SS may consist of both atypical convoluted lymphocytes as well as normal appearing lymphocytes, it may be difficult to ascertain whether Tac staining in these patients is on the neoplastic lymphoid component or the normal infiltrating lymphocytes. Also, the RT-PCR assay used here would not distinguish between p75 expressed in normal or neoplastic cell populations. Because the disease-associated autocrine and paracrine growth stimuli in this disease that support homing to skin and proliferation of neoplastic cells are poorly understood, it is possible that DAB386IL-2 toxin could potentially target not only IL-2R–bearing neoplastic lymphocytes, but also nonneoplastic activated T lymphocytes in patients who experienced PR or BE. Of particular interest in this regard is patient no. 7, who had notable response in the Tac(+) skin (Fig 2), and no response in the Tac(−) Sezary cell population.

Also of importance in the current study was the lack of response in patients whose tumors clearly demonstrated expression of one or more components of the IL-2R (Table 2). Presence of the high-affinity IL-2R complex, measured in this study by immunohistochemical staining for Tac and expression by RT-PCR of p75, may not be sufficient in Sezary cells for successful internalization through the receptor because of possible inherent defects in receptor internalization in patients’ tumor cells. This defect may be related to expression or function of the p64 or γ-chain of the IL-2R, shown to be necessary for internalization events through the receptor. Supporting the concept of a potentially defective IL-2R are reports that, although Sezary cells may express IL-2R, they cannot uniformly be induced to proliferate in vitro in the presence of IL-2. Alternatively, the lack of meaningful response in the patients with detectable but lesser degrees of Tac expression (Table 2) may reflect the expected decreased efficacy of the agent if the tumor is heterogenous and a significant portion of the tumor cell population is truly Tac(−).

Despite the lack of effect of DAB386IL-2 on numbers of circulating Sezary cells in the treated patients, the selective enrichment after three courses of therapy of a neoplastic clone bearing both CD3-TCRαβ and CD25 in patient no. 2 (Fig 4) suggests a modulating effect of the fusion toxin on a circulating tumor cell CD25(+) population. The decrease in sIL2-R in 4 patients, although also not always clearly related to response, may also reflect activity of the fusion toxin to decrease directly tumor burden or to modulate secretion of the soluble form of the receptor by “normal” T cells (Table 3).

Pharmacokinetic data generated in this study (Table 4) and in prior phase I studies using DAB386IL-2 suggest that the agent has a short half-life and rapid clearance. In addition, our studies showed reduced AUC with an increase of anti–DAB386IL-2 antibody titer. This finding exactly recapitulates the findings of preclinical experiments in rats, in which induction of anti-DT antibodies was associated with lower peak level and increased clearance of DAB486IL-2.
response data, because refractory patients have been shown to be less likely to respond to other biologic therapies, including interferon (compare Kohn et al and Vegna et al). Earlier stage patients may be expected to retain features of normal T-cell physiology that would predict responsiveness to T-cell growth factor receptor-directed therapies. The lack of significant toxicity particularly to myeloid cells encourages also the application of fusion protein toxins predicated on IL-2 and IL-2R to early stage patients either alone or in combination with other active agents.

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Chimeric fusion protein toxin DAB486IL-2 in advanced mycosis fungoides and the Sezary syndrome: correlation of activity and interleukin-2 receptor expression in a phase II study

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