



[haematologica reports]
2006;2(13):57-60

Optimizing denileukin diftitox (Ontak®) therapy

M. DUVIC

*Professor of Medicine and Dermatology,
Deputy Chairman, Dept of Dermatology,
The University of Texas M. D. Anderson Cancer Center,
Houston, TX, USA*

Denileukin diftitox (Ontak®) is a genetically engineered recombinant fusion protein composed of the full length interleukin 2 and the catalytic domain of diphtheria toxin. As such, Ontak or formerly DAB389IL2 is the first targeted therapy of the fusion toxin class to be approved. This therapy has broad applicability for targeting any ligand receptor that internalizes after binding. Ontak was first introduced in 1992, and tested for safety and activity in autoimmune disease (rheumatoid arthritis, psoriasis), type I diabetes, HIV, and hematologic malignancies (Strom TB 1993). The initial fusion protein DAB486IL2 initially tested (LeMaistre 1993) was modified to DAB389 which is cleared more slowly and has longer half-life of 72 minutes. On the basis of a randomized two arm, Phase III multi-center clinical trial, Ontak received FDA approval in 1999 for cutaneous T-cell lymphoma (CTCL) (Olsen 2001). Since that time Ontak has been studied and shown efficacy in peripheral T-cell lymphomas, Non-Hodgkin's B cell lymphoma, chronic lymphocytic leukemia, graft-vs-host disease and against T-regulatory cells present in solid tumors.

Targeting the T-cell receptor

Cutaneous T-cell lymphomas are extra-nodal non-Hodgkin's lymphomas that present in the skin but can progress to systemic lymphomas, transform to large cell lymphomas, or become leukemic. The most commonly encountered CTCL are mycosis fungoides (MF) and its leukemic variant, the Sèzary syndrome (SS) (Vonderheid 2002). MF arises from accumulation of skin homing (epidermotropic) helper/memory T-cells which may be stimulated by chronic antigens. T-cells that encounter antigens or superanti-

gens through their T-cell receptor are activated and upregulate the expression of the IL-2 receptor. This stimulates cell division and a clonal T-cell expansion. Clonal expansion of T-cells can be detected by polymerase chain amplification of the T-cell γ or β variable chain regions. Patients who have the same clonal rearrangement of their T-cell receptors from different sites or over time, are more likely to have disease progression over time (Vega 2002).

In the majority of patients, MF begins as an indolent and persistent eczematous or psoriasiform dermatitis which can be treated by skin directed modalities. However, in patients who progress or transform, aggressive chemotherapy treatment can further compromise the immune system and can lead to fatal infection. Thus, for this disease, therapy targeting only the malignant T-cells specifically would represent an advance in treatment strategy.

Waldman first targeted the IL-2 receptor with anti-Tac antibody and showed that the IL-2 receptor is expressed on activated and HTLV-1 infected T-cells but not on resting T and B cells (Waldmann 1993). Ontak is somewhat similar to targeted antibodies in that it binds to the receptor. But Ontak is a ligand for the receptor, and once it binds is internalized with high and intermediate affinity IL-2 receptors found on the surface of activated T-cells. The high affinity T-cell receptor is formed by a trimer of three distinct protein chains: CD25 (α , p55), CD122 (β , p75) and CD132 (γ , p 65). Ontak can bind to any of the receptors but only the intermediate (β, γ) and the high affinity receptors are able to internalize Ontak, which is essential for its cytotoxicity. Following binding of Ontak, the IL-2 receptor plus fusion protein is internalized by endocytosis. The active fragment of diphtheria tox-

in is released into the cytosol following cleavage and inhibits protein synthesis through ADP-ribosylation and causes cell death (Nichols 1997).

Clinical trials

In the first Phase I multi-center dose escalation trial, DAB389IL2 (Ontak, denileukin diftitox) was administered to 109 patients with hematologic malignancies (LeMaistre 1998). In this study, expression of IL-2 receptor subunits was required for entry and was detected in patients' lesions using immunohistochemistry staining. CTCL patients, compared to patients with Hodgkin or with NHL had a higher number of patients with the receptor detected by staining for p55 (CD25) or p75 (CD122) subunits (LeMaistre 1998). Seventy-three patients (44 men and 29 women), aged 16 to 81 years (mean, 50.7) with CTCL (n = 35), NHL (n = 17), and HD (n = 21) who had failed 0 to 15 previous therapies (median, 4) were treated. Ontak was administered as short intravenous infusions daily for 5 days and courses were repeated every 21 days. Patients received one to six courses (mean, 3.3) of Ontak with doses of 3 to 31 µg/kg/day. Asthenia was the dose limiting toxicity at a maximum tolerated dose of 27 µg/kg/day. Half of all patients had significant antibody titers to diphtheria toxin or to DAB389IL-2 at the time of enrollment compared with 92% with titers at the end of treatment but antibodies did not block clinical responses. In CTCL patients, five complete (CR) and eight partial (PR) remissions were observed for an overall response rate of 38% among 32 CTCL patients (Nichols 1997; Saleh 1998). One CR and two PR were seen in patients with NHL and none among patients with HD. Median time to response was 2 months and duration of response was 2 to 39+ months (LeMaistre 1998).

The observation of efficacy and complete responses seen in CTCL patients was the basis for the design of two larger multicenter randomized Phase III trials for registration of denileukin diftitox (DAB389IL-2, Ontak) [Ligand Pharmaceuticals Inc, San Diego, CA, USA]. One Phase III study in early patients who had received less than three prior therapies included randomization to a placebo arm and has been slow in accruing patients. The other Phase III trial was successfully conducted in patients with stage IB to IVA CTCL who had received three or more prior therapies with a median of 5 (Olsen 2001). Patients required biopsy-proven CTCL expressing CD25 on > or = 20% of lymphocytes. Patients

were randomized to receive infusions of either 9 or 18 µg/kg/d of denileukin diftitox administered 5 consecutive days every 3 weeks for up to 8 cycles. Overall, 30% of 71 CTCL patients had an objective response (20% partial responses and 10% complete responses). Response was measured by the Skin Weighted Assessment Tool (SWAT) and required two visits three weeks apart to confirm response. Many other patients had clinical responses but left the study early for infusion reactions and did not have responses confirmed at two visits as required by the protocol. The response rate and duration of response based on the time of the first dose of study drug for all responders (median of 6.9 months with a range of 2.7 to more than 46.1 months) were not statistically different between the two doses. However, in patients with tumor stage disease (IIB) the response rate was 38% at the higher dose level compared to 10% at the lower dose ($p=0.07$). The overall response rate in early patients (stage I) was 43% at 9 µg/kg/day and was 33% at the higher dose level. One patient with stage IA disease treated on study achieved a complete remission for the past five years following Ontak therapy (Carretero-Margolis 2003).

CTCL is a disfiguring tumor which is highly symptomatic in many patients. As part of the trial, overall quality of life was assessed at each cycle using the Functional Assessment of Cancer Therapy-General (FACT-G) questionnaire, skin appearance (7-point scale), and pruritus severity (10-cm visual analogue scale). Composite FACT-G and most individual subscale scores (physical, social/family, emotional, and functional well being) in documented responders (n = 21) gradually increased during the study period and generally reached statistical significance ($P < 0.05$) by cycle 3. The scores of the responding patients were significantly ($p < \text{or} = 0.041$) higher than the scores of nonresponders at endpoint. Responders also assessed their skin severity and pruritus as having significant improvement ($p < \text{or} = 0.05$) at study endpoint compared to baseline (Duvic 2002).

Side effects of Ontak

In this study, premedications were limited to acetameniphen and benadryl and steroids were not given such that many patients experienced infusion reactions. The severity and number of side effects were not associated with dose level of Ontak. Adverse events consisted of flu-like symptoms (fever/chills, nausea/vomiting, and myalgias/artralgias), and acute infusion-related

events (hypotension, dyspnea, chest pain, and back pain) were seen in 69% of the patients and were responsible for 29% of patients leaving the trial early. Chest and back pain and shortness of breath were relieved by slowing the rate of infusion. A delayed vascular leak syndrome (hypotension, hypoalbuminemia, edema) was experienced in 27% of the patients at about 10 days after the first infusion, and generally did not recur. Hypoalbuminemia occurred in 79%, including 15% with grade 3 or 4 changes. Sixty-one percent of patients experienced transient elevations of hepatic transaminase levels with 17% grade 3 or 4. Hypersensitivity rashes were seen uncommonly in some patients, but could be followed by clearing of skin lesions. There was no evidence of cumulative toxicity and no association between response and formation of antibodies. Infection rate was not increased and no myelosuppression was seen.

Using Ontak in the outpatient setting is safe and can be given through peripheral lines. Acute allergic reactions which are rare as well as fever and chills can be blocked by giving low doses of prednisone (10-20 mg) or decadron prior to the infusion. The use of steroid premedication in a small study of 15 patients was associated with increased reported response rates of 60% and is suggested to induce expression of CD25 on T cells. Only three of 15 patients experienced infusion reactions and 2 had vascular leak syndrome (VLS). Saline infusion and hydration following Ontak infusions also appears to dramatically reduce the incidence of VLS. Bexarotene has also been administered prior to Ontak and may also be associated with increased IL-2 expression and possible synergy. As part of this study we also observed one case of thyrotoxicosis presenting as infusion related tachycardia and hypertension. Additional patients were observed with transient thyroiditis and subsequent hypothyroidism suggesting the need for monitoring thyroid function in patients receiving Ontak (Ghori 2006). Visual changes including blindness have recently been reported in six patients.

Importance of IL-2 receptor in determining response to Ontak

Reactivity to CD25 was variable among biopsies taken from the same patient from different lesions and over the course of the study and patients who had lost CD25 staining were able to respond to Ontak. Since CD25 only stains for the alpha chain and it is possible that low numbers of IL-2 receptors may not be detectable, it is pos-

sible that patients with a low level of IL-2 receptors on their malignant cells will respond. In order to study the value of CD25 immunoreactivity for predicting response to Ontak, we prospectively stained lesional skin biopsy specimens from 113 mycosis fungoides and Sezary Syndrome patients for activation markers CD25 and CD30 (Talpur 2006). We proposed to correlate expression with clinical tumor-node metastasis (TNM) stage, histologic grade, and response to denileukin diftitox. High expression was defined as positivity of > or =20% of lesional T-cells using immunohistochemistry (IHC).

We found that CD25 and CD30 expression was more common among lesions from advanced patients ($p = 0.04$ and 0.002 , respectively) and that advanced TNM (T3 or T4) was significantly associated with intermediate-grade ($p = 0.002$) and large-cell transformation histology ($p = 0.04$). Clinical responses to Ontak were observed in 78.5% of patients with high CD25 expression versus only 20% with low to undetectable CD25 expression ($p = 0.01$) among 24 patients who were treated with standard 5-day infusions of denileukin diftitox at $18 \mu\text{g}/\text{kg}/\text{day}$. Split biopsies examined by the reference lab had discordant findings with our assay in over half of the patients. These data suggest that high CD25 expression by IHC is associated with advanced CTCL and with clinical response to denileukin diftitox therapy. However, a multicenter trial has also been conducted to assess this same question (Foss *et al*, in press). Some of the issues relating to detection are the threshold level of receptor expression, difficulty in obtaining representative tissue, lack of an assay that accurately reflects high-affinity receptor, and the potential difficulty of observer variability in evaluating the assays (LeMaistre 2000).

Ontak in other indications:

Frankel et al treated patients with recurrent or refractory chronic lymphocytic leukemia (CLL) (Frankel 2006). Denileukin diftitox administered as 60-minute intravenous infusions for 5 days every 21 days at a dose of $18 \mu\text{g}/\text{kg}$ per day for up to 8 courses produced complete remission in 1 of 22 (4%) patients who received at least two cycles and partial remission in 5 of 22 patients (23%) for a total remission rate of 27%. Twelve of 22 patients achieved reductions of peripheral CLL cells, with 5 of 12 patients achieving >80% reductions. Six of 22 patients achieved reductions in the size of lymph node on examination and computed tomography scans, and all 6 of those patients met the criteria for a partial or complete response that lasted > or = 2 months.

Bone marrow complete remission lasted for 1 year in 1 patient. Progression-free intervals in the responders were 2 months in 2 patients and 4 months, 6 months, 7 months, and 12 months in 1 patient each. Toxicities were moderate and no infections associated with immunosuppression were seen. We treated a patient with tumor stage MF and CLL who had a two year complete remission of the CLL after receiving several courses of Ontak therapy for his CTCL. This indication deserves further study as it is not immunosuppressive or myelosuppressive.

Non-Hodgkin's B-cell Lymphoma

In the original Phase I/II Trial, 3 NHL patients responded, two of whom had follicular lymphomas, and a third having a primary intermediate-grade B-cell NHL refractory to chemotherapy and stem cell transplant. This patient has remained in complete remission over 3 years after treatment with DAB(389)IL-2 (LeMaistre 2000). Dang *et al.* conducted a Phase II trial in 45 patients with refractory NHL at MD Anderson Cancer Center (Dang 2004). Thirty-two of the 45 patients (71%) were refractory to their last chemotherapy. Patients received Ontak on the standard dosing protocol of every three week five day courses and were followed carefully for signs of VLS. The overall response rate was 24.4% with 3 complete responses (6.7%), 8 partial responses (17.8%) and 9 patients with stable disease. Median time to treatment failure was 7 months and progression free survival at 20 months was 24% (95% C.I. 0-60%). In this study there was no difference in responses seen in patients with high CD25⁺ staining (22%) and low CD25 reactivity (29%).

Graft-vs-host disease and autoimmune disease

Targeting IL-2R-expressing lymphocytes may be an effective strategy for the prevention of graft rejection and to treat or prevent graft-versus-host disease (LeMaistre 2000). In a small study 20 patients were treated at a low dose of 4.5 µg/kg/day on days 1-5 and then weekly day 8, 15, 22, and 29. At day 36 there were 9 of 20 complete responses and at day 100, there were 6 responses, four of which were complete (Shaughnessy 2005).

DAB(389)IL-2 has been examined in clinical trials of psoriasis and rheumatoid arthritis and has shown promising results. The potential utility in other autoimmune disorders is unknown, but diseases such as systemic lupus, scleroderma, and vasculitis also may be effective candidates for such ligand fusion therapy (LeMaistre 2000).

References

- Carretero-Margolis CD, Fivenson DP. A complete and durable response to denileukin diftitox in a patient with mycosis fungoides. *J Am Acad Dermatol* 2003;48: 275-6.
- Dang NH, Hagemester FB, Pro B, McLaughlin P, Romaguera JE, Jones D, et al. Phase II study of denileukin diftitox for relapsed/refractory B-Cell non-Hodgkin's lymphoma. *J Clin Oncol* 2004;22: 4095-102.
- Duvic M, Kuzel TM, Olsen EA, Martin AG, Foss FM, Kim YH et al. Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with denileukin diftitox (ONTAK). *Clin Lymphoma* 2002;2: 222-8.
- Frankel AE, Surendranathan A, Black JH, White A, Ganjoo K, Cripe LD. Phase II clinical studies of denileukin diftitox diphtheria toxin fusion protein in patients with previously treated chronic lymphocytic leukemia. *Cancer* 2006;106: 2158-64.
- Ghori F, Polder KD, Pinter-Brown LC, Hoff AO, Gagel RF, Sherman, et al. Thyrotoxicosis after denileukin diftitox therapy in patients with mycosis fungoides." *J Clin Endocrinol Metab* 2006;91: 2205-8.
- LeMaistre CF. DAB(389)IL-2 (denileukin diftitox, ONTAK): other potential applications. *Clin Lymphoma* 2000;1 (Suppl 1): S37-40.
- LeMaistre CF, Craig FE, Meneghetti, C McMullin, B Parker, K Reuben, et al. (Phase I trial of a 90-minute infusion of the fusion toxin DAB486IL-2 in hematological cancers." *Cancer Res* 1993; 53: 3930-4.
- LeMaistre CF, Saleh MN, Kuzel TM, Foss F, Platania L, Schwartz G et al. Phase I trial of a ligand fusion-protein (DAB389IL-2) in lymphomas expressing the receptor for interleukin-2. *Blood* 1998;91: 399-405.
- Nichols J, Foss F, Kuzel TM, LeMaistre CF, Platania L, Ratain MJ, et al. Interleukin-2 fusion protein: an investigational therapy for interleukin-2 receptor expressing malignancies *Eur J Cancer* 1997;33(Suppl 1): S34-6.
- Olsen E, Duvic M, Frankel A, Kim Y, Martin A, Vonderheid E, et al. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J Clin Oncol* 2001;19: 376-388.
- Saleh MN, LeMaistre CF, Kuzel TM, Foss F, Platania L, Schwartz G, et al. Antitumor activity of DAB389IL-2 fusion toxin in mycosis fungoides. *J Am Acad Dermatol* 1998;39: 63-73.
- Shaughnessy PJ, Bachier C, Grimley M, Freytes CO, Callander NS, Essell JH, et al. Denileukin diftitox for the treatment of steroid-resistant acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2005;11: 188-93.
- Strom TB KV, Murphy JR, Nichols J, Woodworth TG. Interleukin-2 receptor-directed therapies: antibody-or cytokine-based targeting molecules. *Annu Rev Med* 1993;44: 343-53.
- Talpur R, Jones DM, Alencar AJ, Apisarnthanarax N, Herne KL, Yang Y, et al. CD25 expression is correlated with histological grade and response to denileukin diftitox in cutaneous T-cell lymphoma. *J Invest Dermatol* 2006;126: 575-83.
- Vega F, Luthra R, Medeiros LJ, Dunmire V, Lee SJ, Duvic M, and Jones D. Clonal heterogeneity in mycosis fungoides and its relationship to clinical course *Blood* 2002;100: 3369-73.
- Vonderheid EC, Bernengo MG, Burg G, Duvic M, Heald P, Laroche L, et al. Update on erythrodermic cutaneous T-cell lymphoma: Report on the International Society for Cutaneous Lymphomas. *J Am Acad Dermatol* 2002;46: 95-106.
- Waldmann T. 1992 Stohlman Memorial Lecture: targeting the IL-2 receptor. *Leukemia* 1993;7(Suppl 2): S151-6.