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# Interleukin-2 Fusion Protein: An Investigational Therapy for Interleukin-2 Receptor Expressing Malignancies

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**$DAB_{389}$IL-2 is an interleukin-2 receptor (IL-2R) specific fusion protein with a molecular weight of 58 kD containing the enzymatic and translocation domains of diphtheria toxin (DT) and human IL-2. This fusion protein is able to direct the cytocidal action of the DT enzymatic region only to cells which bear the IL-2R. The human IL-2R exists in three forms: low, intermediate and high affinity. The high-affinity form is believed to be the biologically relevant form on mature, activated T-lymphocytes, B-lymphocytes and monocytes. $DAB_{389}$IL-2 is able to bind selectively to the high-affinity IL-2R in a concentration-dependent manner, and once bound is internalised via receptor-mediated endocytosis. Upon acidification of the formed vesicle, the enzymatic portion of the fusion protein is believed to pass into the cytosol where it ultimately inhibits protein synthesis by inactivation of elongation factor-2, resulting in cell death. The constitutive expression of the IL-2R on certain leukaemic and lymphomatous cells of T and B cell origin has been reported to occur in patients with chronic lymphocytic leukaemia, cutaneous T cell lymphoma (CTCL), Hodgkin's disease and non-Hodgkin's lymphomas (NHLs). A multicentre $DAB_{389}$IL-2 dose-escalation study of patients with IL-2R expressing lymphomas has been conducted. A 10-fold range of doses were evaluated on a five-daily dose schedule. Patients received up to six courses, with an additional two courses permitted for patients with partial responses that appeared to be still improving after six courses. Most adverse experiences were transient and mild. Preliminary assessment of response indicated five complete responses (CR, duration ongoing at 20, 11, 7, 5 and 4 months) and seven partial responses (PR, duration 3–20 months) in the 35 patients with CTCL. One CR (duration > 20 months) in a patient with NHL (Lennett's lymphoma) and two PR (duration 9 and 2 months) in 17 patients with B-cell NHL have been observed. Based on the mode of action of $DAB_{389}$IL-2, its safety profile, and the patient responses associated with the phase I/II clinical trials, a phase III programme in CTCL patients has been initiated and plans for additional trials in NHL patients are targeted for 1996.** © **1997 Elsevier Science Ltd. All rights reserved.**

**Key words: clinical trial, CTCL, $DAB_{389}$IL-2, fusion protein, IL-2, IL-2R, lymphoma**
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## INTRODUCTION

FUSION TOXINS are recombinant hybrid proteins composed of sequences encoding a targeting ligand such as a growth factor or hormone, and those encoding fragments of diphtheria toxin (DT). These single chain polypeptides are built upon a product template contained within the DT molecule. The DT molecule has three functional domains: the receptor binding domain or targeting ligand which recognises and attaches the molecule to cell surface receptors; the membrane translocation domain which functions like a molecular syringe to release the toxic fragment into the cell cytosol; and the toxic domain which kills target cells by ADP ribosylation of elongation factor 2 and subsequent inhibition of protein synthesis. Genetic replacement of the native receptor binding domain of DT with either a eukaryotic cell receptor-specific growth-factor or a peptide hormone sequence has resulted in the creation of a new class of targeted biological therapeutics which can target specific cell surface receptors on malignant or other disease-causing cells.

$DAB_{389}$IL-2 is an interleukin-2 receptor (IL-2R) specific fusion protein with a molecular weight of 58 kD which is expressed in *Escherichia coli*. Recombinant DNA techniques

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were used to construct the fusion gene which contains the nucleotide sequences for the enzymatically active and membrane translocation domains of DT as well as the sequences for human interleukin-2 (IL-2); the latter replaces the DT receptor-binding domain sequence, resulting in a molecule which is cytotoxic for cells bearing the target IL-2R [1].

*Pre-clinical experience with $DAB_{389}$IL-2 fusion protein*

*In vitro* and *in vivo* laboratory models [2] have demonstrated the sensitivity and specificity of $DAB_{389}$IL-2. Only cell lines that express IL-2R are sensitive to the cytotoxic action of $DAB_{389}$IL-2. $DAB_{389}$IL-2 cytotoxicity is mediated specifically by the high-affinity form of IL-2R; binding to the high-affinity IL-2R complex is at least 60-fold greater than to the low- or intermediate-affinity form of IL-2R. $DAB_{389}$IL-2 mediated cytotoxicity is both time- and dose-dependent. Competing concentrations of human-recombinant IL-2 completely block the cytotoxicity of $DAB_{389}$IL-2, and a rat monoclonal anti-IL-2R antibody, which blocks IL-2 binding to the IL-2R, reduces the cytotoxicity of $DAB_{389}$IL-2 100-fold. In an *in vivo* murine model of IL-2R expressing malignancy, $DAB_{389}$IL-2 effectively slows tumour growth in a dose-related manner and is specifically cytotoxic only for an IL-2R expressing tumour. While pre-immunization of the animals with diphtheria toxoid before treatment diminishes the anti-tumour activity of $DAB_{389}$IL-2, the fusion protein is still able to delay the appearance of tumours and extend survival in some of the treated animals [3].

The human IL-2R exists in three forms: low, intermediate and high affinity. The high-affinity IL-2R is a complex of at least three distinct proteins of 55 kD (p55, Tac, $\alpha$ chain), 75 kD (p75, $\beta$ chain) and the recently described 64 kD (p64, $\gamma$ chain) [4]. The subunits, individually or in specific combinations, comprise low (p55), intermediate (p75/p64) and high (p75/p64/p55) affinity binding sites. The low-affinity receptor is incapable of mediating internalisation of bound ligand [5]. In contrast, the p75/p64 complex alone or in association with the p55 chain slowly internalises bound IL-2 [6, 7]. It is believed that the high-affinity heterotrimer is the biologically relevant form of the IL-2R on mature, activated T cells [8]. Expression of the high-affinity form of this receptor is normally restricted to activated T lymphocytes, B lymphocytes and monocytes [9, 10].

Targeting IL-2R on malignant cells is a strategy that could be used to attain selective cytoxicity for malignant cells over normal cells. The constitutive expression of IL-2R on certain leukaemias and lymphomatous cells of T and B cell origin has been reported to occur in patients with chronic lymphocytic leukaemia, cutaneous T cell lymphoma (CTCL), Hodgkin's disease and non-Hodgkin's lymphomas (NHLs) [11].

*Clinical experience with $DAB_{389}$IL-2 fusion protein*

CTCL is a malignant lymphoma defined by the presence of skin plaques and/or tumours which are immunopathologically characterised by infiltration with malignant T-lymphocytes which have the phenotypic characteristics of CD4 lymphocytes [12]. In approximately 50% of cases, the malignant cells express IL-2R as demonstrated by immunohistochemical staining with anti-CD25 MAbs. Included in the disease spectrum are mycosis fungoides and Sezary syndrome, the latter being a diffuse erythrodema with circulating malignant lymphocytes usually having the phenotype $CD3^+$, $CD4^+$ and $CD7^-$. The natural history of CTCL is best predicted by the stage of disease: patients with only patch/plaque skin disease (stage I, IIa) have a median survival of 12 years; patients with skin tumours, lymph node or blood involvement (stage IIb to III) have a median survival of 5 years; and patients with lymph node effacement, bone marrow or visceral involvement (stage IV) have a median survival of $< 3$ years. The natural history of untreated CTCL has not been characterised systematically since evidence of malignancy is felt to require administration of palliative or possibly curative treatments [13–23].

Despite the chronic nature of these lymphomas, patients experience significant disability as a result of frequent skin infection, disfigurement, pruritus and pain, and thus treatment is required even in the earliest stages. Patients with patch/plaque skin lesions alone are often treated effectively with topical therapies such as nitrogen mustard, psoralen phototherapy or electron beam radiation therapy; however, remission without maintenance therapy is unusual [24]. Patients often experience acute and chronic adverse effects and may eventually become unresponsive to these agents. Moreover, maintenance therapies are frequently cumbersome requiring daily application of topical preparations or numerous visits to the hospital for a period of years and often associated with chronic toxicity. In early stage CTCL, the occurrence of prolonged remissions ($> 8$ years) associated with continued maintenance therapy is approximately 20–30%.

Patients with generalised skin involvement, lymph node enlargement and/or visceral involvement require combination and/or systemic therapies. These therapies (chemotherapy, $\alpha$-interferon and/or retinoids) are variable, applied depending on a given centre's experience [25, 26]. Efficacy of these treatments is inconsistent and often associated with significant short- and long-term adverse effects. In addition, extension of survival does not appear to be influenced by aggressive combination therapy [24]. Thus, there is a critical medical need for an additional and less toxic therapy to manage this malignancy.

Based upon the rationale that malignant cells of certain leukaemias and lymphomas express IL-2R, a phase I/II clinical trial has been conducted to examine the safety and tolerability of $DAB_{389}$IL-2 as a potential treatment for patients with IL-2R expressing malignancies [27]. Of 86 CTCL patients screened for inclusion in this study, 63% were shown to have IL-2R expressing malignant cells by immunohistochemistry. $DAB_{389}$IL-2 was administered as a brief i.v. infusion over 5–15 min once daily for 5 days on an out-patient basis. Repeat courses were administered every 3 weeks to maximum improvement; usually 4–8 courses were administered. Of 35 CTCL patients, five complete responses (CR) (duration ongoing at 20, 11, 7, 5 and 4 months) and seven partial responses (PR) (duration 3–20 months) were observed across a range of dose levels. In addition, most patients experienced significant relief of pruritus, pain and disfigurement occasioned by their CTCL lesions. In 17 heavily pretreated NHL patients, 1 CR (duration $> 20$ months) and 2 PR (duration 9 and 2 months) were observed.

The safety profile of $DAB_{389}$IL-2 has been characterised by the overall experience in 73 lymphoma patients over $> 244$ courses of $DAB_{389}$IL-2, together with experience of 150 patients with severe rheumatoid arthritis, recent-onset insulin-dependent diabetes, HIV infection or psoriasis. The three most common adverse experiences attributed to $DAB_{389}$IL-2 have been fever and chills (75%), transient, non-cumulative, hepatic transaminase elevations (62%), which occurred primarily during the first course of treatment and nausea (53%). Most adverse events were mild and transient.

*Ongoing studies with $DAB_{389}IL$-2 fusion protein*

Therefore, based on the need for a more effective and safe treatment for CTCL and based on the mode of action of $DAB_{389}IL$-2, its safety profile, and the patient responses in the phase I/II clinical trials, a phase III clinical programme, composed of two clinical protocols, has been designed to evaluate the efficacy of $DAB_{389}IL$-2 for second-line treatment in distinct groups of CTCL patients who could benefit from systemic therapy. The first study is a multicentre, double-blind, randomised trial comparing two dose levels of $DAB_{389}IL$-2 in IL-2R positive CTCL patients with recurrent or persistent disease, thereby demonstrating a need for second-line therapy. Enrollment will be stratified by disease stage ($\leq$ IIa; $>$ IIa) and will include patients with stage Ib to IVa disease who have failed standard therapies. Patients will be treated with either 9 or 18 μg/kg/day of $DAB_{389}IL$-2 (32 patients per treatment arm) for five consecutive daily i.v. doses every 21 days for 6 months (eight courses of $DAB_{389}IL$-2). Dosing will continue as long as disease does not progress or for two courses beyond complete response. The primary study endpoint is response rate as determined by tumour burden assessments in all patients using a weighted extent/severity skin core. Supportive study endpoints include assessment of relief of clinical symptoms and duration of response. The second study is being conducted concurrently and is a multicentre, randomised, double-blind, placebo-controlled trial designed to characterise efficacy in patients who either have indolent disease (stages Ia–III), or who have not responded to, or who have relapsed following as many as three standard treatments. Patients are stratified according to disease stage ($\leq$ IIa; $>$ IIa) into one of three groups: placebo, or 9 or 18 μg/kg/day of $DAB_{389}IL$-2 (40 patients per treatment arm). They will be treated for five consecutive daily i.v. doses of $DAB_{389}IL2$ every 21 days for 6 months (eight courses). Again, dosing will continue as long as disease does not progress or for two courses beyond complete response and the endpoints will be as in the other study.


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