Targeting diphtheria toxin to growth factor receptors
John R. Murphy and Johanna C. vanderSpek

Biochemical, genetic and X-ray crystallographic analysis of diphtheria toxin have demonstrated that the native toxin is composed of three structural domains that function in an ordered fashion to intoxicate a eukaryotic cell. With the knowledge that, if delivered to the cytosol, a single molecule of the catalytic domain is lethal for the cell, we have used recombinant DNA methods to genetically replace the native toxin receptor binding domain with a series of growth factors. The resulting diphtheria toxin-related cytokine fusion proteins, or fusion toxins bind to their respective receptors, are internalized by receptor-mediated endocytosis, and efficiently eliminate target cell populations by the adenosine diphosphate ribosylation of elongation factor 2. Based upon the results of preclinical studies, DAB486IL-2, DAB389IL-2 and DAB389EGF have, or are in the process of being evaluated in Phase I/II clinical trials. To date, administration of the diphtheria toxin-based fusion proteins targeted toward the high affinity IL-2 receptor have been found to be safe, well tolerated, and capable of inducing remission in refractory hematologic malignancies.

Key words: cytotoxicity / diphtheria toxin / growth factor / psoriasis / receptor

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Almost 15 years ago we submitted a proposal to the Recombinant DNA Advisory Committee (RAC) to conduct two experiments under Biosafety Level 4 (BL-4). The first experiment was to clone the structural gene for diphtheria toxin in Escherichia coli K-12 and, based upon the assumption that biologically active diphtheria toxin could be expressed in vitro and secreted into the periplasmic space, the second experiment proposed the genetic substitution of the native diphtheria toxin receptor binding domain with the polypeptide hormone α-melanocyte stimulating hormone (α-MSH). The initial experiment was designed to test the feasibility of expressing a potent toxin from a Gram positive organism in a heterologous Gram negative host. The second experiment addressed the possibility of creating ‘new’ toxins that would be selectively active and eliminate only those cells expressing target receptor on their surface. Although these experiments were approved by the RAC, this research was put on administrative hold for almost four years, in part, because of its controversial nature. By the fall of 1985, we were finally allowed to proceed with only those experiments involving the genetic substitution of the native diphtheria toxin receptor binding domain with α-MSH at the National Institutes of Allergy and Infectious Diseases (NIAID) BL-4 facility at Frederick, Maryland.

The diphtheria toxin platform for genetic engineering

The choice of diphtheria toxin as the toxophore for receptor binding domain substitution was based upon a number of earlier observations. In their classic study, Uchida, Gill and Pappenheimer demonstrated that the structural gene for diphtheria toxin was carried by corynebacteriophage β. This study also provided the foundation for subsequent studies on the structure function relationships of diphtheria toxin by demonstrating that the enzymatically active A fragment was positioned on the N-terminal end of the toxin; whereas, the receptor binding domain of the toxin was carried on fragment B. Shortly thereafter, Murphy et al. used β-phage DNA to program S-30 extracts of E. coli and demonstrated, in this coupled transcription translation system, that biologically active diphtheria toxin could be synthesized in vitro.

Using [3H]-Triton X-100, Boquet et al. clearly demonstrated that fragment B of both diphtheria toxin and the non-toxic mutant CRM45 carried a hydrophobic domain which had properties of integral membrane proteins. These investigators postulated that the role of this hydrophobic domain was to facilitate the delivery of fragment A across the eukaryotic cell membrane and into the cytosol. Thus, it was known quite early that native diphtheria toxin was a three domain protein consisting of the enzymatically active domain (fragment A), the hydrophobic
domain (N-terminal portion of fragment B), and the receptor binding domain (C-terminal portion of fragment B).

The process by which diphtheria toxin intoxicates sensitive eukaryotic cells involves at least the following steps: (i) binding of the toxin to its cell surface receptor, (ii) activation of the catalytic domain by a proteolytic cleavage (‘nicking’) of the toxin in a sensitive exposed 14 amino acid loop that is subtended by Cys186 and Cys201, (iii) internalization of the bound toxin into endosomes by receptor-mediated endocytosis, and following acidification of the endocytic vesicle, (iv) the facilitated delivery of the catalytic domain across the endocytic vesicle membrane and into the cytosol. Once delivered to the cytosol fragment A rapidly catalyses the adenosine diphosphate ribosylation of elongation factor 2 which results in the inhibition of protein synthesis and subsequent death of the cell.

Receptor binding domain

The first step in the intoxication process is the specific binding of diphtheria toxin to its cell surface receptor. Middlebrook et al. were able to correlate the apparent sensitivity of a given cell line to diphtheria toxin with the number of receptors on the cell surface. The initial localization of the diphtheria toxin receptor binding domain to the carboxy-terminal region of fragment B was based upon the findings that CRM45, a pre-mature chain termination mutant of the toxin which lacked the C-terminal 15,000 dalton region, failed to block the toxic activity of diphtheria toxin on cells. In addition, observations made by many investigators using different approaches strongly suggested that the functional native receptor binding domain was positioned in the carboxy-terminal 50 amino acids of the toxin.

Processing/activation domain

Once bound to its cell surface receptor, intact diphtheria toxin must be processed into an active two chain protein through proteolytic processing, or nicking. Intact diphtheria toxin has an exposed serine-protease sensitive 14-amino acid loop that is subtended by a disulfide bond between Cys185 and Cys201. As shown in Figure 1, the cleavage domain of the toxin is positioned between the catalytic and transmembrane domains. Nicking, or processing of the toxin may occur either prior to the toxin binding to its cell surface receptor by serum proteases or subsequent to receptor binding by cell surface associated proteases. In either case, it is clear that processing must occur before the catalytic domain is translocated to the cytosol. The protease recognition sequence of diphtheria toxin is Arg190-Val191-Arg192-Arg193 which has been shown to be a site for the endoprotease furin. Additional support for a furin-mediated cleavage of the toxin as an essential step in the intoxication process comes from a site-directed mutational analysis of DAB<sub>486</sub>IL-2, in which destruction of the furin recognition site resulted in a marked loss of cytotoxic activity in the fusion toxin. More recently, it has been shown that furin can be used to specifically nick intact diphtheria toxin in vitro. Tsuneoka et al. have shown that LoVo cells, which do not produce furin are not sensitive to the action of diphtheria toxin; however, upon transfection of LoVo cells with the gene encoding furin, the cells become sensitive to the toxin. Since it has been shown that furin cycles from the trans-Golgi to the membrane, it is almost certain that intact diphtheria toxin, and the diphtheria-related fusion toxins are processed on the cell surface by furin.

Transmembrane domain

It has been known for over a decade that once diphtheria toxin is bound to its cell surface receptor, it is internalized into the cell by receptor-mediated endocytosis. Early endocytic vesicles are known to be acidified by specific vesicular ATPases to an average pH value of 6.2. It is well known that diphtheria toxin must pass through an acidic compartment in order to deliver the catalytic domain to the cytosol. Over a decade ago, it was recognized that under acidic conditions diphtheria toxin and CRM45 will spontaneously insert into the plane of lipid bilayers and form channels. Moreover, the diameter of the channel has been reported to be 18 Å which is large enough for a denatured, extended fragment A to pass through and be delivered to the cytosol. Importantly, diphtheria toxin-induced channels have been observed in both Vero and CHO cell membranes following a low pH-pulse. The channel formation that has been observed in both artificial and cellular membranes involves the insertion of transmembrane domain α-helices. O’Keefe et al. found that the introduction of the E349K mutation, which is positioned in the short loop connecting the transmembrane helices 8 and 9, resulted in both a decrease in the cytotoxic potency and channel-forming activity of the mutant toxin.
These investigators proposed that following protonation of E349, and possibly D325 in the endocytic vesicle, transmembrane helices 8 and 9 would spontaneously insert into the plane of the membrane. Further, upon exposure of the connecting loop to the neutral pH environment of the cytosol, E349 would become deprotonated and its charge would lock, or anchor this α-helical hairpin in the membrane. In an analogous fashion, Falnes et al. reported that the diphtheria toxin-related site-directed mutant D318K.

Figure 1. Ribbon diagram of the X-ray crystal structure of native diphtheria toxin as modified by Bennett et al. The catalytic domain (red), transmembrane domain (magenta), and receptor binding domain (blue) are shown. N, N-terminal end of the toxin; PSL, 14 amino-acid protease sensitive loop which separates the catalytic from transmembrane and receptor binding domains; C, C-terminal end of the toxin. The ribbon diagram was generated using MOLESCRIPT.
[D318 is positioned in the loop connecting transmembrane α-helices 5 and 6] also had reduced cytotoxic potency and impaired ability to form channels in artificial membrane bilayers. vanderSpek et al.28 have shown that an intact transmembrane helix 9 is also required for the selective toxicity DAB389L-2. In-frame deletion mutants that lack the C-terminal portion of transmembrane helix 9 had markedly reduced cytotoxic potency and were incapable of forming stable channels in artificial membranes.

The amino-terminal region of the transmembrane domain is amphipathic and has been shown to be structurally homologous to apolipoprotein A1.29 The introduction of mutations into this portion of diphtheria toxin have been shown to result in mutants with a decreased ability to bind to the diphtheria toxin receptor.30 In contrast, mutations in this region of the fusion toxin molecule do not effect receptor binding affinity; however, they may result in non-toxic mutants in which neither receptor binding activity nor channel formation are impaired.31 Since these mutants bind with high affinity and are capable of forming channels in artificial membranes, they define a hitherto unknown step in the intoxication process.

**Catalytic domain**

Relatively little is known of the molecular details involving the translocation of the catalytic domain of diphtheria toxin through the endocytic vesicle membrane and into the cytosol. Moreover, most of our current understanding of the mechanism by which the catalytic domain enters the cytosol is based upon indirect observations. For example, evidence supporting the hypothesis that the catalytic domain must completely unfold comes from the early observations that the pore formed by insertion of the transmembrane domain into the membrane is approximately 18 Å in diameter; only large enough to allow the passage of a completely unfolded catalytic domain. More recently Wiedlocha et al.32 described the construction of a fusion protein in which acidic fibroblast growth factor (aFGF) was linked to the N-terminus of the catalytic domain. While this fusion protein was as toxic toward Vero cells as the native toxin, the addition of heparin to the assay medium (i.e. heparin induces a tight folding of aFGF) resulted in a complete loss of toxicity. These investigators reasoned that the heparin-induced tight folding of the aFGF component of the fusion protein caused a block in the translocation of the catalytic domain into the cytosol. Perhaps the most compelling evidence in support of complete unfolding of the catalytic domain comes from the observations of Falnes et al.33 Using the X-ray crystal structure of diphtheria toxin,34 Falnes et al.33 showed the introduction of paired cysteine residues in the catalytic domain, which were likely to form disulfide bridges, resulted in the formation of mutants with decreased cytotoxic activity.

While it is clear that the disulfide bond between the catalytic and transmembrane domains (Cys186-Cys201) must be reduced in order to release the catalytic domain, the precise cellular location of where this reduction occurs is unknown. Moskaug et al.35 provided evidence that the reduction of the disulfide bond occurs either when the transmembrane domain becomes buried in the lipid core or when the bond is exposed to the cytosol. More recently, Papini et al.36 demonstrated that the reduction of the disulfide bond occurs after the low-pH insertion of the transmembrane domain into an early endosomal compartment.

Since membrane-impermeant sulfhydryl blockers inhibit the action of diphtheria toxin, it has been proposed that the reduction of the disulfide bond between the catalytic and transmembrane domains could happen either at the level of the cell surface or in the lumen of the endocytic vesicle. In support of this hypothesis, Mandel et al.37 have provided evidence that protein disulfide isomerase (PDI) plays a major role in the diphtheria toxin mediated intoxication of Vero cells. Since PDI has been reported to be primarily localized in the endoplasmic reticulum and direct evidence demonstrating that PDI plays a role in the intoxication process is lacking, this hypothesis awaits further experimental support.

**Diphtheria toxin-related growth factor fusion proteins**

In 1980, we39 began to investigate the role that the hydrophobic domain of fragment B played in the delivery of fragment A to the cytosol of target cells. This study was prompted by the observation that an epidermal growth factor (EGF) ricin A-chain conjugate toxin was exquisitely cytotoxic for cells bearing the EGF receptor; whereas, the analogous conjugate toxin assembled with fragment A of diphtheria toxin was essentially devoid of activity.40 We reasoned that the failure of diphtheria toxin fragment A-based conjugate to be active was likely due to either the lack of a hydrophobic domain that facilitated the entry of the catalytic domain to the cytosol or to stearic
constraints imposed by the EGF portion of the conjugate. Accordingly, we modified the imidazole ring of the central histidine of thyrotropin releasing hormone (TRH; pyro-Glu-His-Pro-NH₂) by the addition of acetylcyctamine. Once modified, acetylcyctaminyl-THR was then coupled through a disulfide bond to either CRM26 (fragment A) or CRM45. While both CRM26-TRH and CRM45-TRH were found to specifically bind to the TRH receptor on CH₃ rat pituitary cells, only the CRM45-TRH conjugate was cytotoxic (IC₅₀ = 3 × 10⁻⁹ M). In marked contrast, CRM26-TRH was devoid of activity at concentrations greater than 10⁻⁷ M. These experiments demonstrated that the hydrophobic domain of fragment B was essential in order to facilitate the delivery of fragment A to the cytosol. The most troubling aspect encountered in the assembly of the CRM45-TRH conjugates was that the specific toxicity (IC₅₀/mg conjugate) varied widely from preparation to preparation. This variation was presumably due to differences in the extent of acetylcyctaminylation of native TRH, differences in either the extent of modification of CRM45 with N-succinimidyl-3-(2-pyridyl)dithio)-propionate (SPDP), and/or in the extent of coupling of the modified proteins into the hormone toxin conjugate.

In order to overcome the uncertainties associated with chemical conjugation, we turned to the methodologies of protein engineering and recombinant DNA to assemble fusion genes in which the native receptor binding domain of diphtheria toxin was replaced with specific ligands. From the outset, the prospect of using recombinant DNA methods to assemble the structural genes encoding bacterial toxin growth factor fusion proteins, or fusion toxins, offered significant advantages over chemical conjugation in the assembly of chimeric proteins. Most importantly, the fusion junction, or point at which the substitute receptor binding domain was linked to the toxin fragment, was precisely determined. Subsequent expression in recombinant E. coli then resulted in the synthesis of a single gene product rather than the mixture of isomeric forms that result from chemical conjugation of two proteins. Thus, we reasoned that by in-frame deletion analysis, it should be possible to determine the diphtheria toxin fragment B (transmembrane domain) structure required for the efficient delivery of fragment A through the membrane and into the cytosol. Furthermore, with the proviso that the distribution of the targeted receptor was limited to subsets of cells that were involved in a pathogenic process, we envisioned that at least some of the fusion toxins might be useful in the treatment of human disease.

Murphy et al.⁷ described the construction and properties of a fusion protein, DAB₄₈₆α-MSH, in which the native diphtheria toxin receptor binding domain was replaced with α-melanocyte stimulating hormone (α-MSH). This construct employed a unique SphI restriction endonuclease site in the diphtheria toxin structural gene such that amino acid 486 served as the fusion junction between diphtheria toxin-related sequences and α-MSH. Despite the fact that DAB₄₈₆α-MSH was subject to marked proteolytic degradation in E. coli, we were able to partially purify sufficient levels of the fusion toxin to demonstrate α-MSH receptor specific toxicity. Importantly, we were able to demonstrate by guinea pig and mouse challenge experiments conducted under BL-4 containment, that the recombinant E. coli expressing DAB₄₈₆α-MSH were avirulent. This data supported the argument that further construction of novel recombinant diphtheria toxin-related cytokine fusion genes could safely be conducted in our laboratory in Boston under BL-3 containment.

Since DAB₄₈₆α-MSH was subject to marked proteolytic degradation, and the protease(s) sensitive sites appeared to be close to the fusion junction between diphtheria toxin and α-MSH, we reasoned that a fusion toxin constructed with a growth factor of larger mass might provide stearic hinderance and thereby minimize degradation. In fact this was found to be the case, and much of our current understanding of the diphtheria toxin-related fusion toxins comes from DAB₄₈₆IL-2 and DAB₃₈₉IL-2 in which the native receptor binding domain of the toxin was replaced with interleukin 2.⁴¹-⁴⁴ As shown in Table 1, a variety of growth factors have been used to replace the native diphtheria toxin structural gene such that amino acid 486 served as the fusion junction between diphtheria toxin and α-MSH. In all instances, these fusion toxins have been shown to be selectively

### Table 1. Diphtheria toxin-based cell receptor-targeted fusion proteins

<table>
<thead>
<tr>
<th>Fusion toxin</th>
<th>Receptor</th>
<th>Cytotoxicity (IC₅₀)</th>
<th>Ref</th>
</tr>
</thead>
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<tr>
<td>DAB₄₈₆α-MSH</td>
<td>α-MSH</td>
<td>n.d.</td>
<td>7</td>
</tr>
<tr>
<td>DAB₃₈₉α-MSH</td>
<td>α-MSH</td>
<td>3 × 10⁻¹¹ M</td>
<td>47</td>
</tr>
<tr>
<td>DAB₃₈₉L-2</td>
<td>IL-2</td>
<td>1 × 10⁻¹¹ M</td>
<td>41</td>
</tr>
<tr>
<td>DAB₄₈₆L-2</td>
<td>IL-2</td>
<td>1 × 10⁻¹² M</td>
<td>64</td>
</tr>
<tr>
<td>DAB₃₈₉L-4</td>
<td>IL-4</td>
<td>2 × 10⁻¹⁰ M</td>
<td>46</td>
</tr>
<tr>
<td>DAB₃₈₉L-6</td>
<td>IL-6</td>
<td>2 × 10⁻¹¹ M</td>
<td>49</td>
</tr>
<tr>
<td>DAB₃₈₉L-7</td>
<td>IL-7</td>
<td>1 × 10⁻¹¹ M</td>
<td>50</td>
</tr>
<tr>
<td>DAB₃₈₉EGF</td>
<td>EGF</td>
<td>1 × 10⁻¹¹ M</td>
<td>48</td>
</tr>
<tr>
<td>DAB₃₈₉D4</td>
<td>HIV gp120</td>
<td>1 × 10⁻⁹ M</td>
<td>45</td>
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</table>
Table 2. Cytotoxicity, binding affinity, and planar lipid bilayer conductance of DAB<sub>389</sub>IL-2 and in-frame deletion mutants (modified from ref 51)

<table>
<thead>
<tr>
<th>Fusion toxin</th>
<th>Cytotoxicity (IC&lt;sub&gt;50&lt;/sub&gt;·10&lt;sup&gt;-12&lt;/sup&gt;Μ)</th>
<th>Binding affinity (K&lt;sub&gt;d&lt;/sub&gt;·10&lt;sup&gt;-12&lt;/sup&gt;Μ)</th>
<th>Conductance (pS)</th>
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<tr>
<td>DAB&lt;sub&gt;389&lt;/sub&gt;IL-2</td>
<td>3</td>
<td>6.6</td>
<td>42</td>
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<tr>
<td>DAB&lt;sub&gt;(D381–387)&lt;/sub&gt;389IL-2</td>
<td>70</td>
<td>8.0</td>
<td>40</td>
</tr>
<tr>
<td>DAB&lt;sub&gt;(D369–387)&lt;/sub&gt;389IL-2</td>
<td>6,000</td>
<td>9.1</td>
<td>33*</td>
</tr>
</tbody>
</table>

*Unstable channels.

...toxic for only those eukaryotic cells which express the appropriate cell surface receptor.7,41-42,45-50 Williams et al42 were able to map the optimal fusion junction by analysis of internal in-frame deletion mutations. The fusion of IL-2 sequences to amino acid 389 of the toxin gave rise to a second generation fusion toxin which was 10-fold more potent then the prototype DAB<sub>486</sub>IL-2. This observation was recently extended by vanderSpek et al51 who demonstrated that the maintenance of an intact transmembrane helix 9 is essential for cytotoxic activity (Table 2). The fusion of IL-2 sequences to amino acid 371 of diphtheria toxin, which is positioned in the middle of transmembrane helix 9, resulted in a fusion protein that was ≥2,000-fold less cytotoxic, and had a markedly reduced ability to form stable channels in planar lipid bilayers compared to the parental DAB<sub>389</sub>IL-2. With the solution of the X-ray crystal structure of diphtheria toxin,34,52 it became clear that the fusion of growth factor sequences to amino acid 389 of diphtheria toxin was optimal. Remarkably, amino acid 389 was at the end of a random coil separating the transmembrane from the receptor binding domain of the native toxin.

**Clinical evaluation of DAB<sub>389</sub>IL-2**

It has been clear for almost a decade that the high affinity form of the IL-2 receptor is an attractive target for cytotoxic therapy in both cancer and autoimmune disease.53,54 Since the high affinity receptor for IL-2 is transiently expressed on both T and B cells and is not found on other normal tissue, therapeutic agents targeted to this receptor offer the possibility of high selectivity, and as a result correspondingly low adverse effects. In addition, since the diphtheria toxin-based fusion toxins mediate their effect through the ADP-riboseylation of elongation factor 2, these agents represent a new class of biologic response modifier. A series of phase I/II clinical studies were developed to determine the safety, tolerability, and pharmacokinetics of DAB<sub>486</sub>L-2 in patients with refractory hematologic malignancies.55-57 These early studies employed DAB<sub>486</sub>L-2 as the experimental therapeutic in order to establish the 'proof of principal' that the diphtheria toxin-based fusion toxins would show some degree of efficacy in the treatment of human disease. In fact, several of these clinical studies were initiated prior to the isolation and characterization of the more potent DAB<sub>389</sub>L-2 form of the fusion toxin. In order to be eligible for study entry, patients had to present with a hematologic malignancy that was refractory to standard treatment regimes. Patients of either sex over the age of 17 years could be treated if they had adequate hepatic and renal function and a Karnofsky performance score of 70% or greater.

The initial clinical trials were designed as a three patient cohort dose escalation in which single and multiple doses of the fusion toxin were administered by intravenous injection, either as a bolus, or 90-minute infusion. The initial dose was 700 ng/kg per day and was escalated to 400 µg/kg per day. Patients were monitored for adverse effects and response and were scored according to National Cancer Institute criteria. Patients with no evidence of disease for at least four weeks were classified as complete responders (CR); patients whose tumor burden decreased by ≥50% for at least four weeks were classified as partial responders (PR); and patients whose tumor burden decreased by ≥50% were classified as minor responders. The intravenous administration of DAB<sub>486</sub>L-2 was found to be well tolerated at all dose levels. The adverse effects were generally mild and included nausea/vomiting, hypersensitivity, fever/malaise/chills, and elevations in serum hepatic transaminases. Renal insufficiency defined the maximum tolerated dose at levels of DAB<sub>486</sub>L-2 above 400 µg/k per day. Importantly, in all instances the adverse effects were transient, not cumulative, and did not preclude repeated administration of the fusion toxin to patients who responded to therapy. In addition, neither changes in lymphocyte function nor lympho-
cyte subset were detected, and patients were not placed at increased risk of opportunistic infection following treatment.

The time course of analysis of DAB_{486}IL-2 concentrations in serum, using a nonlinear mathematical model, showed that the clearance of the fusion toxin followed a one-component model with a t\(_{1/2}\) of approximately 11 minutes at dose levels of 200–400 µg/kg.\(^{58}\) Moreover, the pharmacokinetics of the fusion toxin did not change in a consistent fashion following multiple courses of administration.

In many patients increased serum levels of soluble IL-2 receptor (sIL-2R) were detected; however, there was no correlation between the clearance rates of the fusion toxin from circulation, and the level of sIL-2R. Bacha (personal communication) had found previously that the presence of 50,000 units of sIL-2R per ml, failed to inhibit the cytotoxic action of DAB_{486}IL-2 in vitro. Following administration of DAB_{486}IL-2, approximately 60% of the patients had an anamnestic response to the diphtheria toxin component of the fusion protein. Few patients, however, had anti-IL-2 titers prior to study entry. After one or more course of fusion toxin administration approximately half of the patients developed low titers of anti-IL-2 antibodies. As was seen with sIL-2R, the presence of either anti-diphtheria toxin-related or anti-IL-2 antibodies did not appear to prevent an anti-tumor response. In fact, the clinical experience with the monoclonal antibody OKT3, and the analysis of neutralizing and non-neutralizing monoclonal antibodies, clearly demonstrated that only those antibodies that effectively blocked binding were neutralizing antibodies.\(^{59-61}\)

Since the native diphtheria toxin receptor binding domain is replaced in the construction of the fusion toxins, it was anticipated that pre-existing anti-diphtheria toxoid antibodies would not interfere with the action of DAB_{486}IL-2 in vivo.

The results of the phase I/II clinical evaluation of DAB_{486}IL-2 has proved in principal that this fusion toxin is safe, well tolerated, and may induce durable remissions in refractory hematologic malignancies. Based upon these studies, the second generation IL-2R targeted fusion toxin, DAB_{389}L-2 has been evaluated in the clinic in a focused phase II study in cutaneous T-cell lymphoma. As shown in Table 3, the intravenous administration of DAB_{389}L-2 to patients with refractory disease has resulted in a remarkable rate of complete and partial remission. In fact, the phase III trial of DAB_{389}L-2 for this indication has recently been initiated.

### Summary and conclusions

A number of years ago we began to ask the fundamental question of whether or not diphtheria toxin could be used as a platform for the development of genetically engineered toxins, in which substitution of the native receptor binding domain with specific growth factors would result in a family of biologically active fusion proteins. These ‘new’ toxins would combine the potent cytotoxic activity of diphtheria toxin with the cell receptor specificity of the growth factor employed as the substitute receptor binding domain. Over the past decade we have learned that the structural genes encoding these fusion toxins could be readily assembled, and that their respective fusion proteins were efficiently expressed and purified in a biologically active conformation from recombinant E. coli. The assembly of these chimeric proteins at the level of the gene has allowed for a detailed analysis of structure function and to an increased understanding of the mechanism by which the catalytic domain of diphtheria toxin is delivered to the target cell cytosol. Most importantly, however, the first of these fusion toxins, DAB_{389}L-2, has shown remarkable promise as an experimental therapeutic in the treatment of IL-2 receptor positive hematologic malignancies. The intravenous administration of this agent has proved to be safe, well tolerated, and to induce durable remission from disease in a heavily pretreated subset of refractory patients. More recently, this fusion toxin has also been shown to be a safe and effective agent in the treatment of severe psoriasis.\(^{62}\) In fact, the receptor specific action of this fusion toxin has helped to further unravel the underlying basis of psoriasis as an autoimmune, rather than a keratinocyte-based disease. This latter observation opens the possibility that DAB_{389}L-2 may also be useful in the treatment of those autoimmune diseases in which activated proliferating T cells play a major role in pathogenesis.
Acknowledgements

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