Minireview

Targeted Toxins

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Abstract

Targeted toxins, consisting of tumor-selective ligands coupled to polypeptide toxins, represent a new class of cancer therapeutics that kills malignant cells by inactivating cytosolic protein synthesis and inducing apoptosis. A number of these molecules have been produced under good manufacturing practice conditions and given systemically to patients with a variety of neoplasms. The promising results to date and the remaining pharmacological hurdles are discussed.

Introduction

Targeted toxins consist of a targeting polypeptide covalently linked to a peptide toxin. The targeting protein may be an antibody or antibody fragment, such as a single-chain antibody. These reagents are called immunotoxins. When the targeting moiety is a cytokine, growth factor, or peptide hormone, the molecule is referred to as a fusion protein toxin. The targeting protein or ligand directs the molecule to a cell surface receptor or determinant; the toxin moiety then enters the cell and induces apoptosis by, in most cases, inactivating protein synthesis. Extremely potent catalytic toxins that can kill cells with as few as 1 molecule/cell are found in plants, bacteria, and fungi. The toxins most commonly modified for the construction of targeted molecules that have been clinically evaluated include DT[2] and PE from bacteria and ricin and Gel and PAP isolated from plants.

The two initial challenges in synthesis of a clinically effective targeted toxin are: (*a*) to identify a ligand that will selectively target to every malignant cell in the body; and (*b*) to modify the toxin so that it will no longer bind normal tissues. The ligand and modified toxin are then covalently linked together.

Recently, a number of ligands have been found that bind with high affinity to antigens or receptors on neoplastic tissues. These are listed in Table 1. None of the targets are truly tumor specific. In fact, most are differentiation antigens or growth factor receptors. Nevertheless, initial clinical data suggest that in certain cases, there may be more target on the surface of cancer cells, or that the loss of particular normal tissues in patients bearing the target does not produce serious side effects. One would expect the rapid overgrowth of tumor cells lacking the target, but these "resistant" relapsing tumors have not been commonly observed to date in clinical trials of these agents (31). An additional note is that the ligand after binding the target must internalize by receptor-mediated endocytosis to permit the toxin to gain entry to the cytosol. Many targets have had to be abandoned prior to *in vivo* or clinical testing because they lacked this crucial property. Nevertheless, a number of targeted toxins have been made that bind to and internalize tumor cells in tissue culture in a selective fashion. Once produced in sufficient quantities, many of these have been tested clinically. The encouraging results with targeting the IL-2 receptor, the B-cell differentiation antigen CD22, and local targeting of transferrin receptors in brain tumors suggest that sufficient biological delivery can be obtained for clinical benefit.

Toxins must be modified, both to remove normal-tissue binding sites and for linkage to the tumor-selective ligand. DT is a M_r 58,000 protein with an NH_2-terminal ADP-ribosylation catalytic domain that inactivates cellular protein synthesis, a hydrophobic middle domain responsible for translocation of the ADP-ribosylation domain to the cytosol, and a COOH-terminal cell binding domain (32). DT has been genetically modified for targeted toxin synthesis by either a point mutation in the binding domain, altering a critical Ser-525 to a phenylalanine (CRM107; Ref. 33), or deletion of the 147-amino acid residue cell-binding domain (DT388 or DAB_{389}; Ref. 34). PE is a M_r 68,000 polypeptide with an NH_2-terminal cell-binding domain, followed by the amphipathic helix-containing translocation domain and a COOH-terminal ADP-ribosylation domain (35). PE has been altered to eliminate normal tissue binding by deleting amino acid residues 1–252 and 365–384, yielding PE38 (36). Ricin toxin is a M_r 64,000 heterodimer consisting of an A subunit with rRNA *N*-glycosidase enzyme activity that blocks cell protein synthesis linked by a disulfide bond to a B lectin subunit, which binds to normal mammalian cell surface oligosaccharides (37). Ricin is prepared for targeted toxin synthesis by reduction and removal of the cell-binding B subunit so that only the A subunit (RTA[2]) is used (38) or by chemically blocking the lectin sites of the B subunit with reactive glycopeptides to create a bR molecule (39). Both Gel and PAP are also rRNA *N*-glycosidases capable of inactivating protein synthesis (40). However, they lack cell-binding moieties and hence can be used directly in targeted toxin constructions. Currently, there is insufficient information to choose one toxin over another in the assembly of a targeted toxin for a particular malignancy.

The ligand and modified toxins are linked together, either

Received 10/4/99; revised 11/4/99; accepted 11/4/99.
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[2] The abbreviations used are: DT, diphtheria toxin; PE, *Pseudomonas* exotoxin; RTA, ricin toxin A; bR, blocked ricin; Gel, gelonin; PAP, pokeweed antiviral protein; IL, interleukin; dgA, deglycosylated ricin A chain; HCL, hairy cell leukemia; CTCL, cutaneous T-cell lymphoma; CLL, chronic lymphocytic leukemia; HD, Hodgkin's disease; ATL, acute T-cell leukemia; Tf, transferrin; DTGM, —; GM-CSF, granulocyte/macrophage-colony stimulating factor; MRI, magnetic resonance imaging; VLS, vascular leak syndrome; Mab, monoclonal antibody.

Table 1 Ligands used in clinically tested targeted toxins[a]

Ligand	Receptor/antigen	Toxin	Linker	Name	Ref.
B3 Mab	Lewis^y	PE	C	LMB-1	(1)
B3 sFv	Lewis^y	PE	G	LMB-7[b]	
B3 sFv	Lewis^y	PE	G	B96(sFv)PE40	(2)
B3 dsFv	Lewis^y	PE	G	LMB-9	(3)
RFB4 Mab	CD22	Ricin	C	IgG-RFB4-dgA	(4)
RFB4 Fab′	CD22	Ricin	C	Fab′-RFB4-dgA	(5)
RFB4 dsFv	CD22	PE	G	BL22	(6)
HD37 Mab	CD19	Ricin	C	IgG-HD37-dgA	(7)
B43 Mab	CD19	PAP	C	B43-PAP	(8)
Anti-B4 Mab	CD19	Ricin	C	Anti-B4-bR	(9)
IL2	IL2R	DT	G	ONTAK[c]	
Anti-Tac Mab	CD25	PE	C	Anti-Tac-PE	(10)
RFT5 Mab	CD25	Ricin	C	RFT5-dgA	(11)
Anti-Tac sFv	CD25	PE	G	LMB-2	(12)
Tf	TfR	DT	C	HN66000	(13)
454A12 Mab	TfR	Ricin	C	454A12-rA	(14)
IL4	IL4R	PE	G	IL4(38-37)PE38KDEL	(15)
HuM195 Mab	CD33	Gel	C	HuM195-rGel	(16)
GM-CSF	GM-CSFR	DT	G	DTGM	(17)
N901 Mab	CD56	Ricin	C	N901-bR	(18)
TGFα	EGFR	PE	G	TP40	(19)
EGF	EGFR	DT	G	DAB_{389}EGF	(20)
791T/36 Mab	M_r 72,000 antigen	Ricin	C	Xomazyme-791	(21)
260F9 Mab	M_r 55,000 antigen	Ricin	C	260F9-rA	(22)
Ber-H2 Mab	CD30	SO6	C	Ber-H2-SO6	(23)
T101 Mab	CD5	Ricin	C	T101-RTA	(24)
H65 Mab	CD5	Ricin	C	H65-RTA	(25)
XMMME-001 Mab	Proteoglycan	Ricin	C	XMMME-001-RTA	(26)
OVB3 Mab	Ovarian antigen	PE	C	OVB3-PE	(27)
3A1 Mab	CD7	Ricin	C	3A1-dgRTA	(28)
TXU Mab	CD7	PAP	C	TXU-PAP	(29)
e23 dsFv	erbB-2	PE	G	Erb-38	(30)

[a] G, genetic fusion; C, chemical conjugate; SO6, saporin; EGF, epidermal growth factor; TGFα, tumor growth factor α; sFv, single-chain Fv antibody; dsFv, disulfide-stabilized sFv; TfR, Tf receptor; EGFR, epidermal growth factor receptor; GM-CSFR, GM-CSF receptor; Lewis^y, a carbohydrate antigen; CD5, CD7, CD19, CD22, CD25, CD30, and CD56, "cluster-designation" cell surface antigens; erbB-2, Her-2/*neu* gene product; Fab′, Fab antibody fragment.

[b] L. H. Pai-Scherf, D. Pearson, R. Wittes, M. C. Willingham, and I. Pastan. A Phase I study of LMB-7, [B3(Fv)PE38], a recombinant single-chain immunotoxin for advanced solid tumors, submitted for publication.

[c] E. Olsen, M. Duvic, A. Frankel, Y. Kim, A. Martin, E. Vonderheid, B. Jegasothy, G. Wood, M. Gordon, P. Heald, A. Oseroff, L. Pinter-Brown, G. Bowen, T. Kuzel, D. Fivenson, F. Foss, M. Glode, A. Molina, E. Knobler, S. Stewart, K. Cooper, S. Stevens, F. Craig, J. Reuben, P. Bacha, and J. Nichols. Pivotal Phase III trial of two dose levels of DAB389IL2 (ONTAK) for the treatment of cutaneous T-cell lymphoma, submitted for publication.

chemically or genetically, and the conjugates or fusion protein toxins are purified, usually by chromatographic separations. Once purified, selective cytotoxicity to malignant cells has been demonstrated with a number of these molecules, both in tissue culture and animal models. Subsequently, many of these were produced and purified under good manufacturing practice conditions for clinical evaluation.

A number of clinical trials have been conducted with immunotoxins and fusion protein toxins over the last 16 years. These studies defined a number of pharmacological and toxicological barriers that needed to be overcome. Table 2 lists these clinical studies as well as current clinical trials of targeted toxins with a description of the compound, the diseases for which it is being tested, the response rate, and toxicities, where known. Excitingly, the first of the targeted toxins has received Food and Drug Administration approval for human use, and many additional agents are showing significant anticancer activity in Phases I and II clinical studies, primarily in patients with chemotherapy-refractory cancers. The original rationale for the production and testing of these reagents was that they had a different mechanism of action than DNA- or cell division-damaging therapeutics and thus might be effective either alone or in combination in patients with chemotherapy-resistant malignancies. This rationale appears to be at least partly vindicated. Examples of targeted toxins with ≥30% complete and partial remission rates are LMB-2 for refractory HCL, HN66000 for interstitial therapy of high-grade resistant gliomas, and ONTAK (DAB_{389}IL2) for refractory CTCLs. BL22 and DTGM appear promising because they have shown antitumor activity in a large percentage of patients, and maximum tolerated doses have yet to be defined.

Efficacy

Dramatic anticancer efficacy has been seen in five targeted toxin clinical trials. ONTAK, composed of the catalytic and transmembrane domains of DT fused to human IL-2, has received Food and Drug Administration approval for sale. The

Table 2 Recent clinical targeted toxin trials[a]

Agent	Disease	Response	Toxicities	Ref.
ONTAK	CTCL	22/71	VLS[b]	
HN6600	Gliomas	9/15	CNS	(13)
LMB-2	HCL	4/4	Liver	(12)
B43-PAP	ALL	9/19	VLS	(8)
IgG-RFB4-dgA	NHL	6/25	VLS	(41–43)
Fab′-RFB4-dgA	NHL	5/14	VLS	(5)
Ber-H2-SO6	HD	5/12	VLS	(23)
TP40	Bladder cancer	8/43	None	(19)
Anti-B4-bR	NHL	8/59	Liver	(9, 44)
H65-RTA	CTCL	4/14	VLS	(25)
3A1-dgRTA	T-LGL, T-ALL	2/11	VLS	(28)
$DAB_{486}IL2$	CTCL	4/19	Liver	(45)
IgG-HD37-dgA	NHL	3/32	VLS, acrocyanosis	(7)
LMB-1	Carcinomas	2/38	VLS	(1)
Xomazyme-791	Colon cancer	2/16	VLS	(21)
T101-RTA	CLL	2/18	VLS	(24)
Anti-CD25-dgA	HD	2/15	VLS	(11)
N901-bR	SCLC	1/21	VLS	(18)
260F9-rA	Breast cancer	1/9	Schwann, VLS	(22, 46)
XMMME-001-RTA	Melanoma	1/22	VLS	(26)
Erb-38	Carcinomas	0/8	Liver	(30)
OVB3-PE	Ovarian cancer	0/23	CNS	(27)
LMB-7	Carcinomas	0/54	Liver, kidney[c]	
Anti-Tac-PE	ATL	0/4	Liver	(10)
454A12-rA	Adenocarcinoma	0/20	CNS	(14)
454A12-rA	Leptomeningeal cancer	0/8	Arachnoid	(47)
$DAB_{389}EGF$	Breast cancer	0/43	Liver	(20)
BR96(sFv)PE40	Breast cancer	TE		
LMB-9	Carcinomas	TE		
DTGM	AML	TE		
BL22	HCL, CLL, HD	TE		
TXU-PAP	TALL	TE		
IL4(38-37)PE38KDEL	Gliomas	TE		
HuM195-rGel	AML	TE		

[a] AML, acute myeloid leukemia; TALL, T-cell acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; SCLC, small cell lung carcinoma; T-LGL, T-cell large granular lymphocyte leukemia; TE, too early; CNS, central nervous system.

[b] E. Olsen, M. Duvic, A. Frankel, Y. Kim, A. Martin, E. Vonderheid, B. Jegasothy, G. Wood, M. Gordon, P. Heald, A. Oseroff, L. Pinter-Brown, G. Bowen, T. Kuzel, D. Fivenson, F. Foss, M. Glode, A. Molina, E. Knobler, S. Stewart, K. Cooper, S. Stevens, F. Craig, J. Reuben, P. Bacha, and J. Nichols. Pivotal Phase III trial of two dose levels of DAB389IL2 (ONTAK) for the treatment of cutaneous T-cell lymphoma, submitted for publication.

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other four are still being studied, but their clinical activity warrants description.

ONTAK ($DAB_{389}IL2$), administered 9 or 18 μg/kg/day daily for 5 consecutive days every 3 weeks for up to eight cycles, yielded a 30% objective response rate in 71 patients with refractory stage IB–IV CTCLs.[3] The median response lasted 6.9 months, with a range of 2.7–23.7 months. Patients must have shown ≥20% CD25-positive lymphocytes within a biopsy specimen by immunohistochemistry. Among patients with advanced disease (stage ≥IIB), responses were more common at the higher dose (38% *versus* 10%, $P = 0.07$). Decreased tumor burden correlated with amelioration of symptoms, measured by a quality of life questionnaire. In addition, 52% of patients classified as having stable disease showed a 50% decrease in tumor burden at some point during the study, but for <6 weeks. Photographs before and after treatment of one of the complete responders are shown in Fig. 1.

LMB-2 [anti-Tac(Fv)-PE38] was administered to four 2-CdA- and IFN-refractory HCL patients (12). One patient received 63 μg/kg every other day for three doses for a total of two cycles. He had a complete remission, with no evidence of progression after 13 months. A second patient received 30 μg/kg every other day for three doses for a single cycle. The patient had a 95% reduction in leukemia burden by day 30 but could not be retreated because of infections. A third patient received 40 μg/kg for three doses over 6 days and showed a 95% reduction in leukemic cells but developed high titer neutralizing antibodies. A second cycle was given to this patient, but in the presence of antibody, no detectable LMB-2 was

[3] E. Olsen, M. Duvic, A. Frankel, Y. Kim, A. Martin, E. Vonderheid, B. Jegasothy, G. Wood, M. Gordon, P. Heald, A. Oseroff, L. Pinter-Brown, G. Bowen, T. Kuzel, D. Fivenson, F. Foss, M. Glode, A. Molina, E. Knobler, S. Stewart, K. Cooper, S. Stevens, F. Craig, J. Reuben, P. Bacha, and J. Nichols. Pivotal Phase III trial of two dose levels of $DAB_{389}IL2$ (ONTAK) for the treatment of cutaneous T-cell lymphoma, submitted for publication.

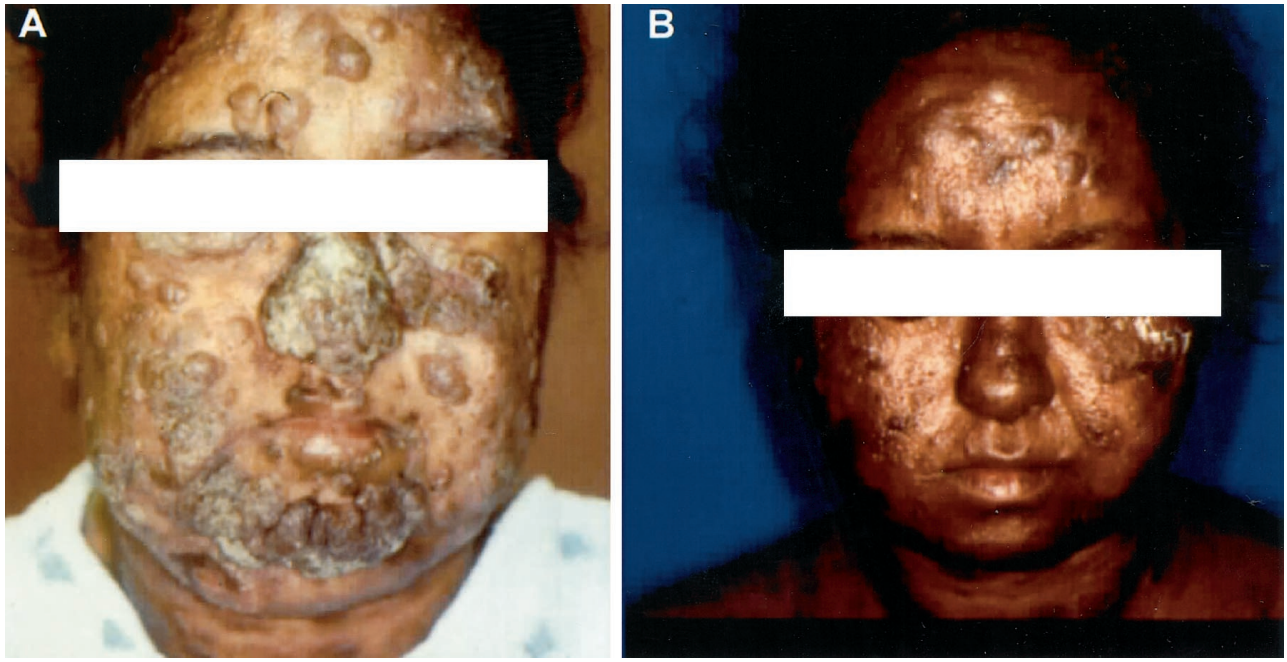

Fig. 1 Photograph of female with IVA CTCL of 2 years duration treated previously with IFN-α and 13-*cis*-retinoic acid with involvement of tumors, patches, and plaques covering 88% of her body surface area and 20% CD25 positive. She received 18 μg/kg/day × 5 and had a partial remission (68% decrease in tumor burden) lasting 5 months duration. *A*, pretreatment; *B*, posttreatment cycle 3. Figure and information provided by Dr. Madeleine Duvic, M. D. Anderson Cancer Center, and used with the permission of the patient.

observed in the circulation, and there was no further response. The fourth patient received a single infusion of 63 μg/kg of LMB-2, and no further agent was given because of diarrhea and reversible cardiomyopathy. A 99% reduction in leukemia cell burden occurred by day 22. Fig. 2 is a graph of the normal and leukemic blood cell counts over time after treatment with LMB-2. LMB-2 has also induced partial responses in patients with CLL, CTCL, HD, and ATL.

HN66000 (Tf-CRM107; CRM107 is a Tf-binding site mutant DT) was infused interstitially via catheters implanted into the tumor beds of patients with high-grade gliomas (13). Fifteen evaluable patients were treated with 0.1–1 μg/ml conjugate infused over 2–16 days with a total volume of 5–120 ml. A 60% partial and complete response rate has been observed, with median survival of responding patients exceeding 1 year. Gadolinium-enhanced T1-weighted MRI scans of one of the complete responders are shown in Fig. 3.

BL22 [anti-CD22(Fv)-PE38] has produced a complete remission in one HCL patient at 10 μg/kg every other day for three doses given in two cycles (6). Both eradication of circulating CLL cells and shrinkage of peripheral adenopathy have been observed.

In some of the other targeted toxin trials, responses have been observed in chemotherapy-refractory patients but at rates of <30%. RFB4-dgA produced a 25% response rate when given by continuous infusion or intermittent bolus schedule in patients with heavily pretreated B-cell non-Hodgkin's lymphoma (4, 42). These included 1 complete remission and 9 partial remissions of 32 patients. RFB4-dgA also produced an unmaintained complete remission of >3 years in a posttransplant lymphoma (43). HD37-dgA (anti-CD19-dgA) produced a lower response rate (~10%; Ref. 7), and the combined anti-CD22 (RFB4-dgA) plus anti-CD19 (HD37-dgA) toxins, referred to as Combotox, when administered at 10, 20, or 30 mg/m^2 over 192 h by continuous infusion yielded a 9% partial response rate with remissions lasting 1 and 5 months.[4] N901-bR (anti-CD56-bR) given at 5–40 μg/kg/day for 7 days by continuous infusion produced 1 partial remission in 21 refractory small cell lung cancer patients (18). LMB-1 (anti-Lewis^y-PE38) was administered by single i.v. bolus infusion at 10–100 μg/kg to 38 patients with advanced solid tumors (1). There was one complete response and one partial response lasting 2 and 7+ months, respectively. RFT5-dgA gave 2 of 10 partial responses in HD (11). Ongoing studies with LMB-9, LMB-1 + Rituximab, HuM195-rGel, DTGM, IL4(38–37)PE38KDEL, TXU-PAP, BR96(sFv)-PE40, and B43-PAP are too early to assess response rates.

Toxicities

In some instances, the targeted toxin receptor/antigen is present on normal tissues, and side effects have occurred. LMB-7 binds Lewis^y antigen on normal stomach mucosa. Initial dose-limiting toxicities at 7–24 μg/kg every other day for three doses were nausea, vomiting, and diarrhea.[5] Endoscopy con-

[4] E. Sausville and R. Messman, unpublished observations.
[5] L. H. Pai-Scherf, D. Pearson, R. Wittes, M. C. Willingham, and I. Pastan. A Phase I study of LMB-7, [B3(Fv)PE38], a recombinant

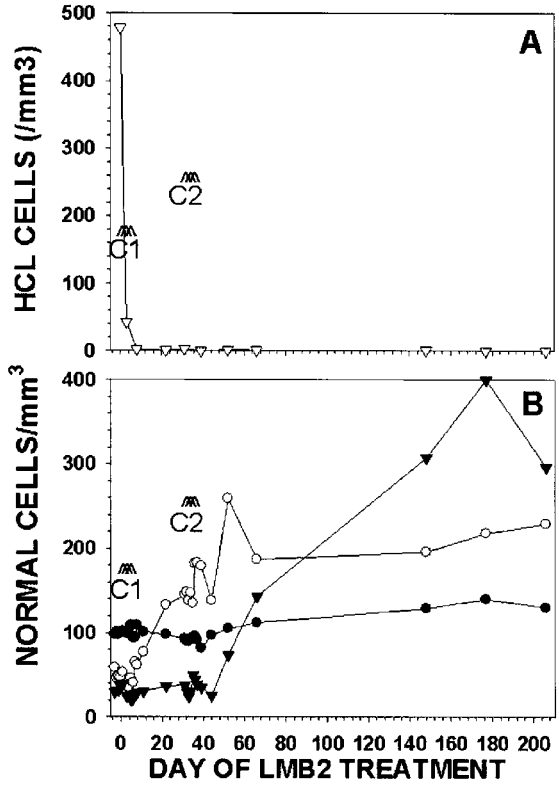

Fig. 2 Response of HCL patient to LMB-2. The patient received LMB-2 at 63 μg/kg i.v. QOD × 3 for two cycles: cycle 1 on day 1 and cycle 2 on day 32. In *A*, the number of HCL is shown: ▽, HCL cells/mm^3. In *B*, normal blood cells are represented by: ▼, absolute neutrophil count/mm^3 × 10^{-1}; ○, platelet count/mm^3 × 10^{-3}; ●, hemoglobin in gm/dl × 10. Figure and legend provided by Dr. Robert Kreitman, NIH (Bethesda, MD), and reproduced with permission (12).

firmed gastritis with apoptotic cells in the body and fundus. Prophylaxis with omeprazole, antiemetics, and loperamide greatly reduced the side effects and permitted dose escalation. DTGM binds the GM-CSF receptor present on mature monocytes, macrophages, and neutrophils. Again, early dose-limiting toxicity was observed at 2–3 μg/kg given daily for 5 days (17). Evidence of cytokine release or systemic inflammatory response syndrome was seen with transient fever, chills, nausea, vomiting, transaminasemia, hypotension, hypoalbuminemia, mild renal and pulmonary insufficiency, edema, and weight gain. One patient was tested and showed a rise in IL-6 and IL-1 receptor antagonist, correlating with symptoms. Once corticosteroid prophylaxis was initiated, these side effects were prevented, and dose escalation continued. HN66000 (Tf-CRM107) binds Tf receptors present on normal brain capillaries. Peritumoral focal brain injury occurred in some infused patients 2–4 weeks after treatment (13). There were stereotypic MRI changes consisting of serpentine strips of increased signal in the peritumoral cortex evident on unenhanced T1-weighted MRI. Biopsy confirmed thrombosed cortical venules and/or capillaries. By lowering the concentration of HN66000 in the infusate and lowering the volume, toxicity to the normal adjacent cortex was ameliorated. Erb-38 bound receptors present on normal hepatocytes and caused hepatotoxicity at low doses (29). OVB3-PE and 260F9-rA reacted with antigen present in the central nervous system and Schwann cells, respectively, and produced dose-limiting neurotoxicities (21, 26).

Toxicities that are independent of ligand have been observed with most targeted toxin clinical studies. These consist of either hepatocyte injury, causing abnormal liver function tests, or vascular endothelial damage with resultant VLS. Both the liver lesion and the vascular lesion may be attributable to nonspecific uptake of targeted toxins by normal human tissues. The normal tissue mediating these injuries may be the tissue showing the observed toxicity, liver or vascular endothelium, or may be macrophages that secondarily release cytokines, producing the liver and blood vessel damage (Fig. 4). There are data supporting both hypotheses. Hepatocytes have been exposed to targeted toxins *in vitro*. The agent binds to the hepatocyte cell surface dependent on the pI of the ligand in the conjugate (48). Separately, animals have been treated with toxins, and macrophage release of cytokines was demonstrated (49). These cytokines, including tumor necrosis factor α, may also directly injure hepatocytes. Clinical proof of which mechanism is more responsible for the transaminasemia in patients treated with fusion toxins is lacking, but it is likely that several mechanisms may operate simultaneously. Nevertheless, the use of targeted toxins with lower nonspecific binding and efforts to block macrophage cytokine release appear warranted. VLS is characterized by weight gain, increased vascular permeability, hypoalbuminemia, myalgias, mild renal and pulmonary insufficiency, and hypotension, and in some cases, aphasias and pulmonary edema. The syndrome occurs transiently, but at times severely, after targeted toxin treatment. VLS usually occurs 4–6 days after initiating therapy and lasts 4–10 days. The cause of the endothelial lesion is unknown. Again, both uptake by the vascular endothelium and macrophages has been postulated as triggering events. There are a number of studies *in vitro* showing endothelial cell culture apoptosis after toxin conjugate exposure (50). Proteins containing a three amino acid motif (x)D(y), where x can be L, I, G, or V and y is V, L, or S appear to bind and damage human endothelial cells *in vitro* (41). This motif has been found in IL-2, PE, and RTA chain and may be partly responsible for VLS. A slight increase in loss of endothelial surface relative to replacement could lead to a significant "leak." Endothelial cells may be uniquely sensitive because of exposure to high concentrations of the targeted toxin in the bloodstream. A correlation between AUC (blood concentration of conjugate × time) and VLS has been reported (4). Smaller fusion toxins or recombinant immunotoxins with shorter circulating half-lives *in vivo* may yield less VLS at comparable doses. Alternatively, inflammatory cytokines released by toxin-ingested macrophages could also produce profound systemic alterations in vascular integrity, as seen with the systemic inflammatory response syndrome. Although no animal model reproduces human VLS, a syndrome of hydrothorax, hypoalbuminemia, hemoconcentration, and neutrophilia developed in rats

single-chain immunotoxin for advanced solid tumors, submitted for publication.

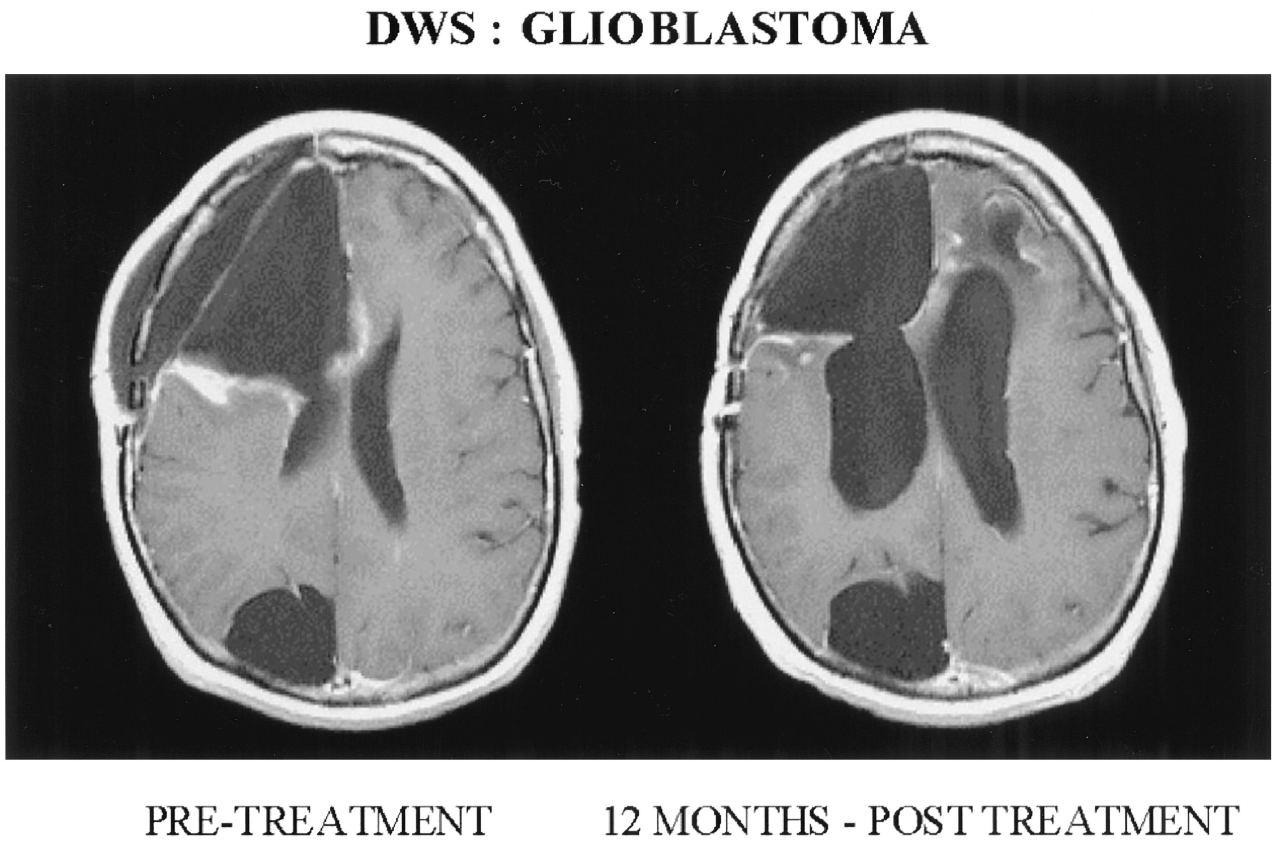

Fig. 3 Gadolinium-enhanced T1-weighted axial MRI scan of female patient DWS with glioblastoma multiforme confirmed by pathology. Initial surgery was in December 1995, when she had whole-brain radiotherapy. She had a recurrence in March 1996 and was treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Because of poor tolerance, she was switched to HN6600 in June 1996. She is still surviving. The first evidence of recurrence was in October 1998, 2 years after agent administration. The recurrence was in the anterior corpus collosum and extended into the left frontal lobe. She also developed hydrocephalus and was treated with a shunt. Figure and information provided by Dr. Sunil Patel, Medical University of South Carolina.

after i.v. injections of anti-Lewis^y Fv-PE40 (51). Rats treated with PE40 alone or IL6-PE^{4E} also developed a VLS-like syndrome (52). The syndrome in rats was prevented by prophylaxis with steroids or nonsteroidal anti-inflammatory drugs. This may be because of the blocking of macrophage cytokine release by these agents. To date, however, there is no clinical data, such as elevated circulating inflammatory cytokines, supporting cytokine-induced vascular trauma. Regardless, it still appears prudent to select smaller targeted toxins. The role of steroid prophylaxis is less certain, because anecdotal reports suggest either "protection" (17) or lack of "protection" in humans (53)as a role.

Other side effects that have been reported with targeted toxins include mild constitutional symptoms when the biological agent is infused rapidly ($<$30–60 min).[3] These symptoms include fever, chills, myalgias, headaches, chest discomfort, and transient hypotension. The infusion may be stopped. The patient may then be symptomatically treated and usually the agent infusion restarted at a slower rate without complications. Corticosteroid prophylaxis appears to prevent these constitutional symptoms. Rarely, patients may have an anaphylactoid response to targeted toxin. Because the agent is protein in nature, rare hypersensitivity is not unexpected. However, the rare occurrence of this complication mandates the administration of the biological agent in a setting where detection and treatment of anaphylaxis are routine. Therapy may include 0.3 ml of epinephrine, 100 mg of Solumedrol, and 25 mg of Benadryl, all given i.v. along with supplemental boluses of i.v. normal saline and nasal prong or face mask oxygen, with continuous cardiac monitoring until resolution. Patients should not be retreated with the same targeted toxin, if anaphylaxis is attributable to the agent, because the reaction is likely to recur with further exposure.

Pharmacology

Recent improvements in disease selection and targeted toxin design has led to an improvement in tumor localization and reduced immunogenicity. However, these remain important pharmacological barriers.

The circulating half-life varied with the size of the molecule. Larger molecules had longer half-lives, with 24 h for Combotox and N901-bR and 9 h for LMB-1 (1, 18, 31). All of these molecules consisted of whole IgGs coupled to peptide

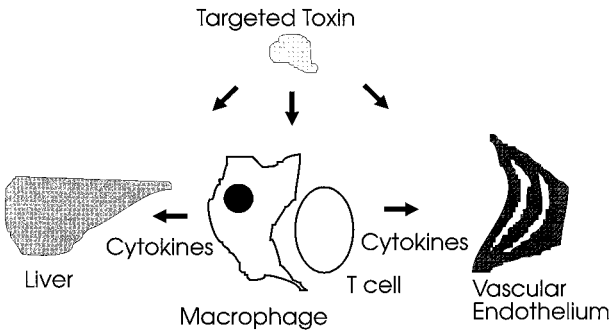

Fig. 4 Mechanisms for targeted toxin liver and vascular side effects. Toxin is either directly taken up by hepatocytes and vascular endothelium, causing transaminasemia and VLS, or toxin is internalized by macrophages. The macrophages then release inflammatory cytokines, which produce liver and vascular damage.

toxins. Smaller molecules, including the single-chain immunotoxins LMB-2 and LMB-7 and the cytokine fusion protein toxin ONTAK ($DAB_{389}IL2$), had shorter half-lives of 5, 2, and 1 h, respectively (12).[3, 5] Some of the clearance of the smaller molecules (M_r ~60,000) may be renal glomerular filtration. However, most of the clearance of these foreign proteins is likely by the liver or reticuloendothelial system.

No clinical protocols have been reported that comprehensively correlated percentage of extravascular tumor cell saturation with dose of targeted toxin. The assumption has been that toxicities, including VLS, hepatotoxicity, or neurotoxicities, prevented sufficient doses to saturate extravascular tumor sites. *In vitro* studies with multicellular tumor spheroids and mathematical models using data from other proteins suggest that smaller-sized fusion toxins and permeability enhancers, such as cisplatin or hyaluronidase, may improve tumor uptake (54, 55). Clinical responses with targeted toxins in lymphomas and leukemias may be attributable, in part, to a significant fraction of circulating malignant stem cells in these diseases.

Targeted toxins generated humoral immune responses in all patients, except those with CLL. Clearly, the development of neutralizing antibodies is detrimental to targeted toxin antitumor efficacy. In many trials, retreatments have been limited to a few cycles because of the development of neutralizing antibodies. Even when the antibodies generated are nonneutralizing, they may form immune complexes and accelerate clearance from the circulation. This antibody response also reduces clinical benefit. Rituximab is a human Mab to the B-cell differentiation antigen CD20. It is being tried in combination with LMB-1 to reduce immune responses to that immunotoxin. Other methods include coadministration of 15-deoxyspergualin or CTLA4Ig and have to date been tried only in animal models (56, 57). Finally, the use of human RNases as the toxophore may be an additional method for reducing conjugate immunogenicity (58).

Conclusions

The potential for targeted toxins as postulated by Paul Ehrlich one century ago (59) has not been fully realized to date. However, over the last 20 years with the advent of genetic engineering and advances in receptor physiology, we have progressed to the point that several targeted toxins have demonstrated clinical utility. Over the next decade, additional ligand-receptor systems should be defined that extend the applications of targeted toxins to additional disease states. Control of the nonspecific toxicities and immune responses with various prophylactic maneuvers should further improve the therapeutic index of these molecules. Finally, combination therapy trials with cytotoxic chemotherapeutic agents are likely to yield even higher response rates with more durable responses, based on preclinical results and clinical studies with other biological/cytotoxic agent combinations. The next decade should see exciting advances in the development of these reagents.

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Clin Cancer Res 2000;6:326-334.