

New Life for Immunotoxin Cancer Therapy

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Abstract

Immunotoxins are targeted anticancer therapeutics that kill cancer cells using a cytotoxic bacterial toxin payload. Their development for use in solid tumor malignancies was delayed due to issues with their immunogenicity and limited therapeutic window. However, new research has rejuvenated the field. Coadministration with a lymphocyte-depleting regimen of pentostatin and cyclophosphamide can delay antidrug antibody formation, increasing the number of treatment cycles that patients can receive and resulting in durable responses in heavily pretreated patients. In

addition, a new generation of immunotoxin molecules with reduced immunogenicity and nonspecific toxicity has been developed through protein engineering techniques, and one has recently entered the clinic. In preclinical studies in mouse models, these new agents are effective against many tumor types as single agents, and also produce synergistic antitumor responses in combination with chemotherapy. These new immunotoxins have renewed excitement in the field and may prove a promising addition to the targeted therapy repertoire. *Clin Cancer Res*; 22(5); 1055–8. ©2015 AACR.

Antibody-based therapies have revolutionized cancer treatment by allowing very specific targeting of cancer antigens. Conjugating cytolytic agents to antibodies allows for specific delivery of these agents to tumors. Antibody-drug conjugates (ADC) carry a chemotherapy drug payload to cancer cells and have demonstrated success in breast cancer and Hodgkin's lymphoma (1, 2). Immunotoxins are very potent molecules that consist of an antibody or antibody fragment linked to a bacterial or plant toxin rather than a traditional chemotherapeutic (3). Once the immunotoxin binds to the target tumor antigen it is internalized, undergoes processing, and ultimately inhibits protein synthesis leading to cell death. Although immunotoxins targeting CD22 have produced complete remissions in refractory hairy cell leukemia and acute lymphoblastic leukemia in children (4, 5), they have been much less effective in targeting solid tumors. One reason for this lack of activity is the development of neutralizing antibodies to the toxin, limiting retreatment of patients. Another is the development of dose-limiting capillary leak syndrome. Because the toxin is a foreign protein it elicits a strong host immune response that limits treatment to one cycle of three doses in most patients with solid tumors. Previous studies using immunosuppressive drugs such as steroids, cyclosporine, single-agent cyclophosphamide, or rituximab did not prevent development of antibodies.

Despite success in treating some hematologic malignancies where the immune system is suppressed, immunogenicity has been a large barrier to the development of clinically useful immunotoxins for solid tumors. Consequently progress in devel-

oping these molecules for solid tumors has been slow. However, our group has recently reported major cancer regressions in patients with mesothelioma treated with an immunotoxin and immune suppression. In addition, we have used protein engineering to generate recombinant immunotoxins that are inherently less immunogenic. These developments could have broad implications for rejuvenating the field of immunotoxin cancer therapy.

Our laboratory uses protein engineering to produce recombinant immunotoxins. These are chimeric proteins that consist of the Fv fragment of an antibody reacting with a cancer cell fused to a truncated form of *Pseudomonas* exotoxin A (PE). Native PE has three functional domains: Domain I, which enables PE to bind to the surface of most cells, Domain II, which enables the toxin to be processed by furin separating the Fv from the toxin, and Domain III, which catalyzes the inactivation of elongation factor 2 leading to inhibition of protein synthesis and cell death (3). Using recombinant DNA technology, we removed Domain I (and additional unnecessary sequences) to produce a truncated PE toxin (PE38) that by itself cannot kill cells. To target the toxin to cancer cells, we replaced Domain I with an Fv selected to react with a cancer cell antigen (6, 7).

Immunotoxins are highly targeted therapies like antibody-drug conjugates, however, using a toxin payload rather than a chemotherapy payload to kill cancer cells results in some unique properties (see Table 1). Most importantly, immunotoxins are ideal to give in combination with standard chemotherapy. Immunotoxins kill cells by irreversibly modifying and inactivating elongation factor-2 to halt cellular protein synthesis, a nonoverlapping mechanism of action from any standard chemotherapy agent. In addition, the main toxicity of immunotoxins, vascular leak syndrome, does not overlap with typical side effects from standard chemotherapies. For this reason, these two classes of drugs can be coadministered in the clinical setting with unmodified dosages of both the chemotherapy and the immunotoxin (8). In preclinical models, this results in synergistic antitumor efficacy (9–11). Another special property of the toxin payload is that it can kill both actively dividing and quiescent cells, since all cells require protein synthesis for survival. This makes it especially critical that the tumor antigens targeted by immunotoxin therapeutics have

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doi: 10.1158/1078-0432.CCR-15-1623

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Translational Relevance

Most anticancer monoclonal antibodies have little cytotoxic activity, but conjugation to tubulin-inhibiting drugs to produce antibody–drug conjugates has proven beneficial in some tumor types. Another approach to make anticancer antibodies therapeutically useful is linking them to potent bacterial toxins that kill tumor cells by inhibition of protein synthesis. These immunotoxins have shown single-agent activity in some hematologic cancers but limited efficacy in solid tumors. The principal reason for their lack of activity is that their immunogenic nature limits retreatment of patients. However, newer immunotoxins generated by protein engineering are inherently less immunogenic and also more active. This increases the likelihood that patients with solid tumors can receive multiple cycles of therapy, resulting in increased antitumor activity. RG7787, a deimmunized immunotoxin targeting mesothelin, has just entered clinical trials for treatment of mesothelin-expressing cancers.

very low or absent expression on normal cells, or expression confined to nonessential tissues, because the toxin also exposes normal cells that express the target antigen to the same cytotoxicity. For example, targeting Her2 with an immunotoxin resulted in severe liver toxicity due to minimal expression of Her2 on normal hepatocytes, while the ADC ado-trastuzumab emtansine is very well tolerated (12). However, this property also makes immunotoxins potentially useful for targeting less replicatively active cancer cell types and tumors that are inherently resistant to chemotherapy, such as hepatocellular carcinoma (13).

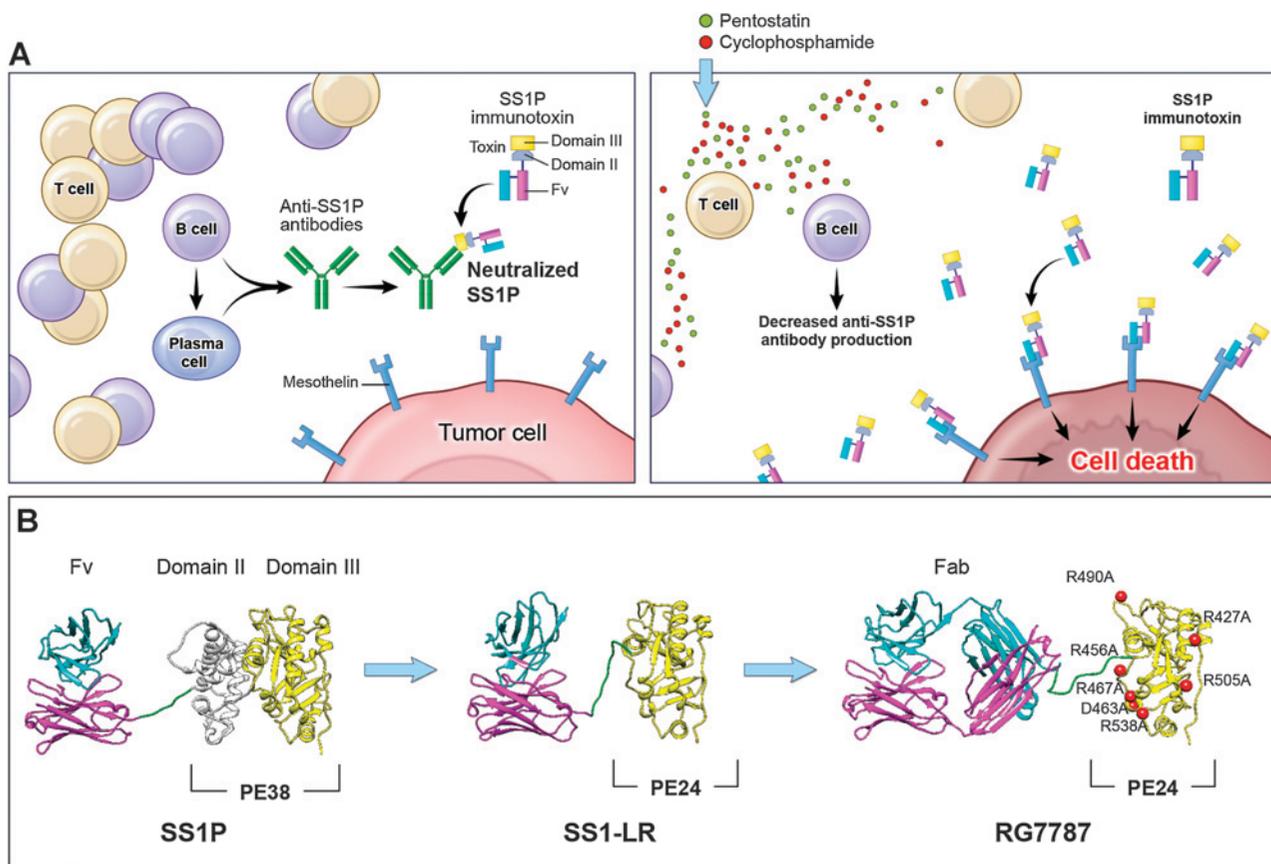
Our current clinical studies are focused on treating mesothelioma with the anti-mesothelin immunotoxin SS1P. Mesothelin is a lineage-restricted cell surface protein present on normal mesothelial cells, but highly expressed in many human malignancies including mesothelioma, and cancers of the pancreas, lung, stomach, ovary and bile duct (14). SS1P consists of an anti-mesothelin Fv linked to PE38. In a phase I clinical trial, SS1P had limited clinical activity by itself. One reason for low activity was that about 90% of patients developed antibodies to SS1P after just

three doses and could not be retreated. Because T and B cells play an important role in antibody production, we evaluated an immunosuppressive regimen that targets both T and B cells to decrease the host immune response against immunotoxins. Using immunocompetent mice, we showed that pretreatment with pentostatin (that kills T cells) and cyclophosphamide (that kills B cells) abolished anti-SS1P antibody formation (15). To evaluate if such a strategy could work in humans, we carried out a pilot study in patients with advanced treatment refractory mesothelioma using pentostatin, cyclophosphamide, and SS1P together. As predicted, this regimen significantly decreased formation of anti-SS1P antibodies allowing more cycles of SS1P to be given (16). Surprisingly, this regimen also induced durable and major tumor regressions in three of the ten evaluable patients with chemorefractory disease. All three patients who had a response were alive more than 2 years from start of therapy. This strategy of combining immunotoxin therapy with pentostatin plus cyclophosphamide could be useful for immunotoxins targeting different tumor antigens and opens up the field of immunotoxin therapy for solid tumors (Fig. 1A).

Because the toxin used to make the immunotoxin is a foreign protein, identifying and mutating its immunogenic epitopes should produce therapeutic molecules that are less immunogenic. We have identified the majority of the B- and T-cell epitopes in PE38 and used protein engineering to remove or silence these epitopes (Fig. 1B). We found that most of Domain II can be deleted from PE as long as 11 amino acids containing the furin-processing site are retained. This deletion removes the B- and T-cell epitopes in Domain II and unexpectedly the mutant immunotoxin (SS1-LR) is usually more active than SS1P in killing cancer cells. It is also about 8-fold less toxic to mice and has a greatly diminished ability to produce capillary leak syndrome in rats (17). Apparently Domain II is responsible for these undesirable properties. To silence B-cell epitopes in Domain III, we mutated seven bulky hydrophilic residues to alanine (18). Because molecules without Domain II are small, about 43-kDa, they are rapidly filtered and removed from the circulation by the kidneys. To increase latency in the blood, we have collaborated with Roche Diagnostics GmbH to produce RG7787 (Ro6927005). This protein has a molecular weight of 72-kDa and contains a humanized anti-mesothelin Fab fused to the mutated toxin (Fig. 1B; Table 2).

Table 1. Comparison of ADC and recombinant immunotoxin therapeutics

	ADC	Immunotoxin
Targeting moiety	Full monoclonal antibody	Antibody fragment
Payload	Chemotherapy drug	Bacterial or plant toxin
Mechanism of action	Most commonly antitubulin agents	Protein synthesis inhibition
Toxicity	Target specific, and most commonly peripheral neuropathy, myelosuppression	Target specific and vascular leak syndrome
Approved drugs in class	Trastuzumab emtansine (for HER-2-positive breast cancer); brentuximab vedotin (for Hodgkin's lymphoma and systemic anaplastic large cell lymphoma)	Denileukin diftitox (for cutaneous T-cell lymphoma)
Advantages	Demonstrated efficacy in refractory setting Better tolerated than most standard chemotherapy	Can kill quiescent and rapidly dividing cells as a single agent Ideal for combination with standard chemotherapy due to nonoverlapping mechanism of action and toxicity profile No off-target toxicity from bystander effect
Disadvantages	Overlapping mechanism of action to standard chemotherapy Cumulative peripheral neuropathy	Immunogenicity Target selection more restrictive because payload is effective against many quiescent normal cells

**Figure 1.**

Avoiding the anti-immunotoxin antibody host response. Decreasing the human immune response to immunotoxins can be accomplished either by manipulating the host immune system or by protein engineering of the toxin moiety. A, after treatment with SSIP alone, patients develop anti-SSIP antibodies made by B and plasma cells. When patients are retreated with SSIP, these preformed antibodies bind to SSIP and prevent it from reaching the tumor (left). Pretreatment of patients with pentostatin and cyclophosphamide before SSIP administration depletes T and B cells and decreases the production of anti-SSIP antibodies (right). This prevents host neutralization of SSIP with repetitive dosing and allows more SSIP to reach tumor cells. B, another approach is to modify the PE toxin to remove immunogenic epitopes so that the toxin is inherently less immunogenic. Structural models of SSIP and its deimmunized variants are shown. The targeting domain consists of V_L (cyan) and V_H (magenta). The linker between the targeting domain and PE contains the furin cleavage site (green), which is required for toxin cytotoxic activity. The furin cleavage site is part of PE Domain II. The remainder of Domain II (gray) is unnecessary for cytotoxicity and has been deleted in the PE24-based toxins, SS1-LR and RG7787. Domain III (yellow) is the catalytic domain of PE. In RG7787, alanine point mutations were introduced at seven bulky hydrophilic residues (red) to silence human B-cell epitopes within this domain. Deletion of Domain II reduces the size of the molecule into the range where it can be easily filtered by the kidneys, reducing serum half-life. RG7787 contains a larger humanized Fab for targeting, which raises its molecular weight above this threshold. This molecule has significant antitumor activity in preclinical models and has now begun phase I clinical testing.

Table 2. Comparison of SSIP and the redesigned anti-mesothelin immunotoxin RG7787

	SSIP	RG7787
Targeting moiety	Anti-MSLN dsFv	Humanized anti-MSLN Fab
PE payload	PE containing Domains II and III (PE38)	PE with most of Domain II deleted and Domain III bearing 7 point mutations to remove B-cell epitopes (PE24)
Payload size	38 kDa	24 kDa
Full molecule size	62 kDa	72 kDa
Activity <i>in vitro</i>	Picomolar range for many tumor cell lines	Picomolar range for many tumor cell lines
Mouse MTD	0.4 mg/kg i.v. every other day $\times 3^a$	3.75 mg/kg i.v. every other day $\times 3^a$
Efficacy in mouse tumor models	Shrinks A431 epidermoid cancer cells expressing transfected MSLN and complete regressions with chemotherapy (9). Ineffective against KLM1 pancreatic. ^a	Decreases tumor volume of MSLN-positive breast (HCC70), gastric (MKN28), and large lung tumors (H596). Cytostatic in KLM1 pancreatic as single agent, but complete regressions with taxane (10, 11, 19)
Immunogenicity	90% of patients make neutralizing antidrug antibodies after 1 cycle (20, 21)	Limited reactivity to antidrug antibodies from sera of patients previously treated with SSIP (18)
Current clinical testing	Phase II in combination studies with pentostatin and cyclophosphamide (NCT01362790)	Phase I for MSLN-positive tumors (NCT02317419)

Abbreviation: MSLN, mesothelin.

^aUnpublished.

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RG7787 is very active against a variety of mesothelin expressing cell lines and is more active than SS1P against cells directly isolated from mesothelioma patients (Hassan; unpublished data). *In vivo*, it has a much larger therapeutic window than SS1P due to the decreased toxicity and therefore improved *in vivo* efficacy even in xenograft models utilizing cell lines with nearly identical *in vitro* sensitivity to SS1P and RG7787. Also, when RG7787 was combined with paclitaxel, it produced complete or near-complete remissions in a pancreatic cancer model in mice (10, 11). RG7787 is currently undergoing phase I clinical testing in patients with mesothelin-positive malignancies including mesothelioma, ovarian, pancreatic, gastric, and triple-negative breast cancers (NCT02317419).

In summary, we have developed new immunotoxins that are designed to be less immunogenic and more active. Second, clinical trial results show that the human antidrug immune response to an immunotoxin can be diminished to allow for repeat dosing. Given on this schedule immunotoxin treatment can produce dramatic tumor responses in some patients. These

efforts herald an exciting development for the field. Larger clinical trials over the next few years will be needed to realize the original promise of immunotoxin therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Hassan, I. Pastan

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Hassan, C. Alewine, I. Pastan

Writing, review, and/or revision of the manuscript: R. Hassan, C. Alewine, I. Pastan

Grant Support

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Received July 8, 2015; revised September 9, 2015; accepted September 30, 2015; published OnlineFirst October 13, 2015.

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Clin Cancer Res 2016;22:1055-1058. Published OnlineFirst October 13, 2015.

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