New Life for Immunotoxin Cancer Therapy

Raffit Hassan¹,², Christine Alewine¹, and Ira Pastan¹

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. Co-administration 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.

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 developing 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
Disadvantages Overlapping mechanism of action to standard chemotherapy

Advantages Demonstrated efficacy in refractory setting
Better tolerated than most standard chemotherapy
Can kill tumor cells that don’t express target through bystander effect

Disadvantages Overlapping mechanism of action to standard chemotherapy
Cumulative peripheral neuropathy

Table 1. Comparison of ADC and recombinant immunotoxin therapeutics

<table>
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<tr>
<th>ADC</th>
<th>Immunotoxin</th>
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<tr>
<td>Targeting moiety</td>
<td>Full monoclonal antibody</td>
</tr>
<tr>
<td>Payload</td>
<td>Chemotherapy drug</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Most commonly antitubulin agents</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Target specific, and most commonly peripheral neuropathy, myelosuppression</td>
</tr>
<tr>
<td>Approved drugs in class</td>
<td>Trastuzumab emtansine (for HER-2-positive breast cancer); brentuximab vedotin (for Hodgkin’s lymphoma and systemic anaplastic large cell lymphoma)</td>
</tr>
<tr>
<td>Advantages</td>
<td>Demonstrated efficacy in refractory setting</td>
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<td></td>
<td>Better tolerated than most standard chemotherapy</td>
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<td></td>
<td>Can kill tumor cells that don’t express target through bystander effect</td>
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<td>Overlapping mechanism of action to standard chemotherapy</td>
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<tr>
<td>Disadvantages</td>
<td>Cumulative peripheral neuropathy</td>
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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 SS1P alone, patients develop anti-SS1P antibodies made by B and plasma cells. When patients are retreated with SS1P, these preformed antibodies bind to SS1P and prevent it from reaching the tumor (left). Pretreatment of patients with pentostatin and cyclophosphamide before SS1P administration depletes T and B cells and decreases the production of anti-SS1P antibodies (right). This prevents host neutralization of SS1P with repetitive dosing and allows more SS1P 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 SS1P 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 SS1P and the redesigned anti-mesothelin immunotoxin RG7787

<table>
<thead>
<tr>
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<th>SS1P</th>
<th>RG7787</th>
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<tr>
<td>Targeting moeity</td>
<td>Anti-MSLN dsFv</td>
<td>Humanized anti-MSLN Fab</td>
</tr>
<tr>
<td>PE payload</td>
<td>PE containing Domains II and III (PE38)</td>
<td>PE with most of Domain II deleted and Domain III bearing 7 point mutations to remove B-cell epitopes (PE24)</td>
</tr>
<tr>
<td>Payload size</td>
<td>38 kDa</td>
<td>24 kDa</td>
</tr>
<tr>
<td>Full molecule size</td>
<td>62 kDa</td>
<td>72 kDa</td>
</tr>
<tr>
<td>Activity in vitro</td>
<td>0.4 mg/kg i.v. every other day (\times 3)</td>
<td>Picomolar range for many tumor cell lines</td>
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<td>Mouse MTD</td>
<td>Shrinks A431 epidermoid cancer cells expressing transfected MSLN and complete regressions with chemotherapy (9). Ineffective against KLM pancreatic.(^*)</td>
<td>Decreases tumor volume of MSLN-positive breast (HCC70), gastric (MKN28), and large lung tumors (H596). Cytostatic in KLM pancreatic as single agent, but complete regressions with taxane (10, 11, 19)</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>90% of patients make neutralizing antidrug antibodies after 1 cycle (20, 21)</td>
<td>Limited reactivity to antidrug antibodies from sera of patients previously treated with SS1P (18)</td>
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<tr>
<td>Current clinical testing</td>
<td>Phase II in combination studies with pentostatin and cyclophosphamide (NCT01362790)</td>
<td>Phase I for MSLN-positive tumors (NCT02317419)</td>
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Abbreviation: MSLN, mesothelin.

\(^*\) Unpublished.
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

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