Commentary

Treatment of solid tumors with immunotoxins

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Abstract

Immunotoxins are hybrid molecules that generally consist of a toxin coupled with a tumor-specific antibody or antibody fragment that is intended to target and concentrate the toxin within the tumor tissue. The biodistribution, specificity, immunogenicity, and cytotoxic activity of the immunotoxin are but a few of the factors that govern the effectiveness of these compounds in the treatment of patients with cancer. Improvements in design, synthesis, and delivery of these molecules may permit them to become significant components in the arsenal of targeted therapies for cancer.

In this issue of *Breast Cancer Research*, von Minckwitz and colleagues [1] report the results of phase I study conducted in patients with advanced cancer using ScFv(FRP5)-ETA, a recombinant, single chain (sc) antibody toxin with binding specificity for HER2 [2,3] linked to *Pseudomonas* exotoxin A (ETA). Although significant clinical responses were not observed, ScFv(FRP5)-ETA appeared to be well tolerated at dose levels that may prove clinically active. As such, ScFv(FRP5)-ETA joins a growing list of promising new agents in targeted therapy for solid tumors.

The notion of targeted tumor killing is has been around since early in the past century. In 1906, Ehrlich [4] introduced the concept of targeting cancer cells with a 'magic bullet', consisting of tissue-specific carriers that would deliver toxic agents to neoplastic tissue. The advent of mAbs made it possible to generate virtually unlimited amounts of antibody specific for antigens that are differentially expressed by tumor cells versus their normal cell counterparts. Although examples of truly tumor-specific antigens are rare, it is often possible to find tumor-associated antigens that are restricted in their tissue distribution or that are expressed at aberrantly high levels by tumor cells. A prime example of the latter is HER2/neu, a surface protein that is over-expressed by many types of cancers, in particular adenocarcinoma of the breast [5-7]. As such, HER2/neu is an attractive target for antibody-directed therapies. In fact, the first mAb approved for the use in solid tumor therapy was Herceptin, a humanized mAb specific for HER2. It is currently used in the treatment of breast cancer as monotherapy or in combination with chemotherapeutic agents. Although remarkable objective responses were observed, only a fraction of patients experienced a complete response and/or a long-lasting response with this mAb [8-11]. In addition, side effects such as cardiac dysfunction have been observed, the incidence and severity of which appears to be greatest in patients receiving Herceptin in combination with anthracyclines [12].

It is conceivable that linking antibodies to HER2/neu with drugs and/or toxins might generate immunotoxins with different mechanisms of action [13,14]. This approach may have lower toxicity because the toxic agent is anticipated to concentrate in and around the tumor, leading to higher local concentrations. Initially, antibodies were conjugated to radioisotopes. Most immunotoxins currently contain modified plant, bacteria, or fungi toxins. The two most commonly used bacterial toxins are diphtheria toxin and *Pseudomonas* exotoxin [15]. An advantage of this approach is that, through selective delivery of drugs to tumors, it can reduce systemic toxicity. One single immunotoxin can kill a tumor cell, whereas 10^5 molecules of a chemotherapeutic drug are needed to achieve the same effect [16].

Another problem that may be circumvented by immunotoxins is that of poor penetration of antibody molecules into some tumor tissues. Through the use of antibody fragments, such as the scFv used by ScFv(FRP5)-ETA, immunotoxins with sizes smaller than antibody molecules can be generated that may achieve more effective penetration of solid tumor tissue than whole antibody molecules [17,18].

von Minckwitz and colleagues [19] previously reported that injection of ScFv(FRP5)-ETA into cutaneous nodules of tumor

 ETA= *Pseudomonas* exotoxin A; mAb = monoclonal antibody; sc = single chain.

cells that over-express HER2/neu caused shrinkage or complete regression of the injected tumor nodules in most treated patients. To develop this into a systemic therapy, those investigators examined the toxicity of intravenous injections of ScFv(FRP5)-ETA in a phase I dose escalation study including 18 patients with advanced cancers that over-expressed HER2/neu. When administered daily as an intravenous bolus infusion over 5 days every 2 weeks, the investigators found that doses of 12.5 μg/kg were well tolerated. Peak plasma concentrations of ScFv(FRP5)-ETA in patients treated at this dose level generally were greater than 100 ng/ml – a level that apparently has activity against tumor cells *in vitro*.

On the other hand, 13 out of the 18 patients treated generated antibodies against ScFv(FRP5)-ETA, which in five cases apparently could neutralize the activity of this immunotoxin. Such antibodies certainly could diminish the plasma concentration of ScFv(FRP5)-ETA with repeated use. Conceivably, the generation of similar immunotoxins containing humanized antibody fragments instead of the mouse anti-HER2/neu antibody fragment could help to mitigate this problem by reducing the tendency for inducing antibodies – that could accelerate the clearance of the immunotoxin and/or inhibit its ability – to bind selectively to their intended tumor target [20].

In any case, on the surface it appears that ScFv(FRP5)-ETA is better tolerated than another Fv immunotoxin directed against HER2/neu, namely erb-38 [21]. The latter immunotoxin combines the same toxin with an anti-HER2/neu Fv via a disulphide bond. However, erb-38 had hepatoxicity apparently at lower doses in phase I testing, an effect that is not completely understood [18]. Unlike erb38, the mAb FRP5 does not bind to liver cells, minimizing the cross-reactivity with normal tissue. This could explain the relatively low toxicity of the agent [19]. However, von Minckwitz and colleagues [1] point out that even in mouse models, in which binding to endogenous HER2 on liver cells can be excluded, erb-38 is still more toxic than ScFv(FRP5)-ETA. Another possibility is that the liver toxicity caused by erb-38 could be due to different clearance in the liver, the binding stability of the toxin moiety, or the difference in the binding epitopes of the two antibodies. These differences should be further explored because a more resilient immunotoxin that lacks systemic toxicity would be very desirable. Such an agent could be administered via continuous infusion to maintain a constant plasma concentration, which might permit optimal perfusion into the tumor mass.

In conclusion, ScFv(FRP5)-ETA is an immunotoxin that is composed of a potent toxin combined with a recombinant single-chain antibody toxin with binding specificity for HER2/neu. Prior studies indicated that direct injection of this immunotoxin into subcutaneous nodules of tumor cells over-expressing HER2/neu could induce local tumor regression. To develop ScFv(FRP5)-ETA for systemic therapy, von Minckwitz and colleagues [1] evaluated the safety of administering this immunotoxin intravenously to patients with HER2/neu over-expressing cancers. Although clinical responses were modest at best and antibodies against ScFv(FRP5)-ETA developed in most of the treated patients, the study found that ScFv(FRP5)-ETA could be administered intravenously to patients at doses that may have clinical activity without major hepatic toxicity. Although more clinical studies are necessary, it is conceivable that ScFv(FRP5)-ETA may find utility in the targeted therapy of solid tumors.

Competing interests

The author(s) declare that they have not competing interests.

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