Genentech's (S. San Francisco, CA, USA)/Biogen-Idec's (Cambridge, MA, USA) Rituxan (rituximab) and Biogen-Idec's Zevalin (ibritumomab) have been approved, which might make it tough to recruit patients for clinical trials. Yet, Vitetta says although these are all great drugs, people relapse, so there is a need for therapies to treat them. "You can't count on one antibody to hold a tumor at bay forever. It just isn't going to happen," she says. And in fact, she has completed a phase 1 trial with a product that is a mixture of immunotoxins, with promising results. Vitetta waxes philosophical when it comes to her role in getting these drugs into the clinic. "I've learned in this business that there's only so much you can do before you have to give up control. Before I let it go, at least we show the company it is a good drug candidate that actually works in humans," she says.

But that's not the end of the story. Vitetta moved her mutated ricin along a parallel trajectory, which has had greater success—a ricin vaccine, which has captured some biodefense funding and is being moved forward as well. By introducing a second mutation that knocks out toxicity of the ricin A chain variant, the Vitetta group created a vaccine candidate that in phase 1 trials was safe and induced antibodies that passively protected mice that were challenged with ricin[2]. They have furthered this project by developing a model for aerosolized and ingested ricin[3], the more likely route from a bioterror attack, and have obtained funds to test the vaccine with adjuvant in a second clinical trial. "If the money holds up, we'll move toward advanced trials with our licensee," she adds.

Ellen Vitetta found that the path to a successful drug can be difficult, expensive and frustrating, but overall worthwhile.

Summing up the years since her 2003 publication, Vitetta comments, "It brought licensing fees from several companies, we moved forward with several grants and learned a heck of a lot about vaccinology and recombinant protein production. Looking back, it was a worthwhile journey and hopefully we will have developed two products that will eventually be approved by the FDA."

1. Smallshaw, J.E. *et al.* Genetic engineering of an immunotoxin to eliminate pulmonary vascular leak in mice. *Nat. Biotechnol.* **21**, 387–391 (2003). (36 citations)
2. Vitetta, E.S. *et al.* A pilot clinical trial of a recombinant ricin vaccine in humans. *Proc. Nat. Acad. Sci. USA* **103**, 2268–2273 (2006).
3. Smallshaw, J.E. Richardson, J.A. & Vitetta, E.S. RiVax, a recombinant ricin subunit vaccine protects mice against ricin delivered by gavage or aerosol. *Vaccine* **25**, 7459–7469 (2007).

Master disseminator

Massachusetts Institute of Technology's (Cambridge, MA, USA) Dane Wittrup is one of the more prolific of those responding to our survey, with five publications in *Nature Biotechnology* in the past ten years. But he harkens back to one of his earliest as having the largest impact on his field of protein engineering. In 1997, he and a graduate student, Eric Boder, described a yeast display system for screening combinatorial libraries[1]. Although not the first protein display technology to be developed—phage display and *Escherichia coli* display had previously been described—this was the first eukaryotic cell–based system, and as such, circumvented certain biases inherent in the prokaryotic protein machinery. In the paper, they describe the construction of a library of fusion proteins of single-chain variable fragments of antibodies to the C-terminus of yeast α-agglutinin, the enrichment of yeast displaying the protein by flow cytometry and the selection from a mutagenized library of variants with slower 'off' times.

And Wittrup says, the method has held up to this day. His laboratory has made some minor improvements over the years, but basically, he says that you can take that 1997 paper and pretty much just follow the methods and you'll have it. In the intervening ten years, though, the technology was spun-out into a company, which stayed virtual until it was bought by BASF (now Abbott; Abbott Park, IL, USA) for $7 million, holder of an exclusive license to the technology, and was packaged into a kit by Invitrogen (Carlsbad, CA, USA), which only just last year discontinued selling it. The kit helped get the technology into people's hands, according to Wittrup, but wasn't a big money maker for the company. All this commercial activity has not deterred researchers from using the technology—in fact the kit was meant to do just the opposite. Consequently, numerous projects by Wittrup and others have led to some successful engineered proteins and a constellation of patents emanating from the original patent.

And that's just the way Wittrup wants it. Although he saw the commercial potential and did his bit to foster that, he feels an ethical duty to disseminate the information as broadly as possible, rather than holding it close for his own financial benefit. What's more, in his view, holding it close doesn't make business sense anyway. "The best thing to do is to get as many people as possible using the technique and seeing how good it is—so that they say this is so good, we have to have it, we have to license it," he says.

This attitude may be why you see more on yeast display than some of the many other protein engineering methods, like mRNA display or *E. coli* display that emerged both before and after yeast display. But Wittrup thinks they are all good, although he has never been motivated to try anything else; yeast display has allowed his group to do everything they wanted to do. Still he comments, "Nobody has a monopoly on making a protein with certain properties." He does admit to a certain partisanship for his platform, however, adding that yeast display may be the best in terms of speed and probability of success. And he points to several converts to yeast display from phage and other systems—Dave Kranz of the University of Illinois, Wittrup's colleague from his days at Illinois, which holds the patents to the technology, and the University of California at San Francisco's Jim Marks.

Dane Wittrup's 1997 method of yeast display holds up to this day.

And where has yeast display taken Wittrup? His group has tackled several interesting human proteins—huntingtin[2], interleukin 2 (ref. 3)—essentially to answer the philosophical question, "Are you just a serial tool builder, or are the tools good enough to use them yourself?" But after making one particular super-binder against a tumor antigen, he found that optimized or not, the antibody wasn't persisting in the tumor, which has led him to ponder questions of pharmacokinetics and biodistribution. "It was a real wake-up call," he says. "We've moved upstream in the discovery process to what's the best way to use this thing."

1. Boder, E.T. & Wittrup K.D. Yeast surface display for screening combinatorial polypeptide libraries. *Nat. Biotechnol.* **15**, 553–557 (1997). (252 citations)
2. Colby, D.W. *et al.* Potent inhibition of huntingtin aggregation and cytotoxicity by a disulfide bond-free single-domain intracellular antibody. *Proc. Natl. Acad. Sci. USA* **101**,17616–17621 (2004).
3. Rao, B.M. *et al.* High-affinity CD25-binding IL-2 mutants potentially stimulate T-cell growth. *Biochemistry* **44**, 10696–10701 (2005)