Henry Daniell says that having a cover photo in two high-impact journals raised the visibility of chloroplast engineering technology.

manufacturing because it would eliminate costly fermentation and purification, associated with other biomanufacturing technologies.

Daniell is the technology founder of two companies; Chlorogen, (St. Louis) which has invested $15 million to develop a proprietary protein therapeutic and Gencrest (Raritan, NJ, USA), which is investing $20 million in phase 1 to advance four chloroplast-derived therapeutics from the Daniell laboratory that have been shown to be functional in animal studies. In keeping with Daniell's personal interest in solving problems in the developing world—he was born in India—GenCrest is working on chloroplast-derived vaccines.

All this sounds good. But with only $20 million in the bank, Daniell knows it's not going to be enough to take any biopharmaceutical product through clinical trials. So even with the imprimatur of venture capitalists, he's still a long way from where he needs to be. But Daniell goes on undeterred—moving antigens for cholera, malaria, amebiasis, rotavirus and tuberculosis into plants, and hoping for the day that these will all make their way to the people who most need them.

1. Daniell, H. *et al.* Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nat. Biotechnol.* **16**, 345–348 (1998). (154 citations)
2. De Cosa, B. *et al.* Overexpression of the *Bt cry2*Aa2 operon in chloroplasts leads to formation of insecticidal crystals. *Nat. Biotechnol.* **19**, 71–74 (2001). (125 citations)
3. Ruhlman, T. *et al.* Expression of cholera-toxin B–proinsulin fusion protein in lettuce and tobacco chloroplasts—oral administration protects against development of insulitis in non-obese diabetic mice. *Plant Biotechnol. J.* **5**, 495–510 (2007).
4. Arlen, P.A. *et al.* Field production and functional evaluation of chloroplast-derived interferon-α2b. *Plant Biotechnol. J.* **5**, 511–525 (2007).
5. Daniell, H. Transgene containment by maternal inheritance: effective or elusive? *Proc. Natl. Acad. Sci. USA* **104**, 6879–6880 (2007).

Timing is everything

In 2002, Ryzard Kole described in our pages a mouse assay for testing antisense oligonucleotides[1]. The assay provides incontrovertible evidence, Kole says, that a particular oligonucleotide works by an antisense mechanism, something that has not always been so clear with antisense. In addition, it allows investigators to track the location and function of oligos injected into whole animals. In his *Nature Biotechnology* paper, Kole and his team designed an oligonucleotide to target a splice variant of green fluorescent protein (GFP). When this oligonucleotide was injected intraperitoneally into mice transgenic for the GFP splice variant, restoration of proper protein splicing and function could be confirmed by the detection of fluorescence in mice tissues, such as colon, small intestine and liver. This elegant assay provided the starting point for Kole to move therapeutic programs forward. But it hasn't been an easy path.

For one thing, although the biology might have been ready back then, the chemistry of the oligos was not. In the intervening years, nucleic acid chemistry has advanced to the point that one can make molecules that are stable *in vivo*. But Kole didn't have that at the time. And now that he does, the world both of researchers and the money men who back them have moved onto sexier therapeutic nucleic acid formats of higher potency, like RNA interference and microRNA.

But Kole remains undeterred, believing that targeting splicing is "a new frontier" and the company he founded back in 2001, Ercole Biotech (Research Triangle Park, NC, USA), is holding its own, according to Scott Forrest, the technology manager at the University of North Carolina, where all this has been playing out. It has space, a staff of three researchers, and a set of collaborations with companies, which is giving it a foothold in the commercial sector. From Forrest's vantage point, the company is independent, sound and secure. "It's a pleasure to see," he says. And without a dime of venture capital, which can be both good and bad, says Forrest. The university's shares in Ercole have not been diluted, but the company doesn't get what venture capitalists bring to the table, in terms of expertise in forming and managing a startup.

Getting there required the university taking the risk in providing early venture money, which has paid off in Ercole's case. "We don't always get it back, but if every once in awhile it leads to a living, breathing company, that's consistent with our larger mission of economic development, an attitude that has helped Research Triangle Park and the region make headway in becoming a biotech hotspot," says Forrest.

Ercole's *raison d'être* is to apply antisense technology to modulating alternative splicing and correcting splicing defects. Some 70% of genes undergo alternative splicing, and a number of serious diseases are caused by errors in splicing. Kole wants to target these diseases, and he's using the assay described back in 2002 to try to get there. To choose what diseases to target, he lets the chemistry be his guide. He says "[you can] see where oligos with a certain chemistry go—which tissues, which cells—and because of that, can chose what is a target gene and a target disease that we know we can treat because that's where the oligo goes."

Earlier this year, Ercole announced a collaboration with the antisense company AVI Biopharma (Portland, OR, USA) for developing therapies for Duchenne's muscular dystrophy and beta-thalassemia. Ercole also has a long-standing deal with Isis (Carlsbad, CA, USA) and a recent agreement with Santarus (San Diego), which affords them access to various oligonucleotide chemistries. Kole says he gets frequent requests from companies and individual investigators to test their oligos and delivery systems.

Ryzard Kole gets many requests for his oligo tracking technology, which he is using to develop therapies for diseases caused by aberrant splicing.

The idea of using antisense to correct splice variants may soon be validated in humans. After the demonstration that systemically delivered oligonucleotides that target splicing have therapeutic effects in a mouse model of Duchenne's muscular dystrophy[2], a Dutch biotech company, Prosensa (Leiden), received permission from the Netherlands Central Committee on Research to initiate a trial in humans using locally administered antisense oligos.

1. Sazani, P. *et al.* Systemically delivered antisense oligomers upregulate gene expression in mouse tissues. *Nat. Biotechnol.* **20**, 1228–1233 (2002). (94 citations)
2. Lu, Q. L. *et al.* Functional amounts of dystrophin produced by skipping the mutated exon in the *mdx* dystrophic mouse. *Nat. Med.* **9**, 1009–1014 (2003).

Too far ahead of the curve?

Some might say John Frangioni was ahead of his time when he published his 2001 article on diagnostic imaging agents to pinpoint osteoblast (bone-forming) activity[1]. Six years have passed, and it's still "the cart before the horse," as he puts it.

The paper describes near-infrared (NIR) fluorescing derivatives of bisphosphonates,