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Innovations

Getting gene therapy under control ARIAD Pharmaceuticals, Inc.

W.A. Wells [1]

1114 First Avenue, Fourth Floor, New York, NY 10021, USA

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If scientists were given free rein in the biotech world they would probably come up with companies like ARIAD Pharmaceuticals, Inc. (Cambridge, MA, USA). Smart ideas, original research, and rigorous papers in high profile journals have been the hallmarks of ARIAD's 8 years of existence.

Clinical trials have, however, been less evident. ARIAD has tackled two tough fields – designing small molecules to alter signal transduction, and creating a regulatory system for gene therapy – and has just recently started its first phase II trial.

If investors are getting anxious, they should probably stay patient for just a little longer. ARIAD's hard work is suddenly paying off in many areas at once. A promising osteoporosis drug enters the clinic in 2001, as do planned trials of the company's most exciting work, which involves small molecule regulation of gene therapy. ARIAD's system may not be needed unless, and until, garden-variety, unregulated gene therapy proves that it can induce sustained and useful expression levels in humans subjects. ARIAD is ready to pounce with their regulation technology as soon as that fateful day arrives. They are hoping that it is soon.

The promise of proximity

ARIAD started out as a signal transduction company. The Src kinase binding osteoporosis drug, and several other drug candidates not discussed in this article, fit this mold. But in November 1993, Stuart Schreiber[2] (Harvard University, Cambridge, MA, USA) and Gerald Crabtree (Stanford University, Stanford, CA, USA) published a paper in *Science* that got ARIAD involved in the expanding field of gene therapy. Schreiber and Crabtree used small molecule dimerizing ligands to bring together proteins (such as growth factor receptors or transcription factors) that are activated by such proximity.

A generic dimerizing system requires that binding sites for the dimerizers be added to the target proteins. To get these modified proteins into the cells of patients requires gene therapy. By 1994, ARIAD's gene therapy program had started.

The planned treatment regimen is an initial dose of gene therapy followed by months or years of pills. If the delivered genes stick around for long enough, the pills will take care of regulating how much protein is produced from those genes.

ARIAD can control gene therapy, but gene therapy itself needs to be proven in the clinic

The treatment is primarily a replacement for injected proteins. In the 1990s, injected proteins were the success story, and gene therapy the sob story. But ARIAD's vice president of gene therapy Tim Clackson says that there are multiple reasons why regulated gene therapy will triumph. Many proteins, he says, have such a narrow therapeutic range that injecting a bolus of protein will lead to toxicity, periods of insufficient therapy, or both. For longer-term treatments, patient compliance with daily injections becomes a problem. Gene therapy offers a once-off injection

[1] *A to A of Biotech.* Innovations started 4 years ago with a column on Affymax, and now finishes 48 companies later with a profile of ARIAD. I've had a great time writing these columns, and thank you for your interest and feedback. Links to all articles in the series can be found at http://www.biotext.com/list.html.

E-mail: wells@biotext.com

[2] Stuart Schreiber is both the editor of this journal and the chairman of ARIAD's board of scientific and medical advisers. This profile was, however, written by a freelance contributor, and neither the choice of the company as a subject nor the content of the article were influenced by any of the editors of this journal.

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that can be localized, but it comes with the same bolus problem, and a concern that there is no way of turning the whole thing off. ''With something (as potent as) Epo (erythropoietin) you absolutely can't have an unregulated gene therapy,'' says Clackson.

Coming ready or not

In November 1993 the logic of the ARIAD approach was already substantially in place. The following 7 years have seen a lot of tinkering, such as the addition of a 'bump' to the dimerizer and a 'hole' to the binding protein, so that the natural version of the protein is not affected by the added drug. Progress, says CEO Harvey Berger, ''may have been a little slower than we would have liked.'' But ARIAD's progress is comparable, he says, to that of other companies tackling new areas, such as Tularik, Inc. (South San Francisco, CA, USA). ''If there is one very clear message, it's that ARIAD took on a very tough area of science. (ARIAD and Tularik) both went down some paths that didn't work out, and ARIAD had the added complexity of gene therapy being one of the areas we are pursuing.''

ARIAD must perfect both gene product and small molecule activator, and prove that both are safe. But it is a third component – the method for delivering the gene – that has been slowing things up. ARIAD has been waiting for gene therapists to make a vector that produces protein for long enough to warrant regulation.

''Some people say we are running before we can walk,'' says Clackson. ''Our feeling is that gene therapy is now a reality, and we want to be poised and ready when the call comes for greater safety and control.''

''It was a fair criticism a couple of years ago,'' continues Clackson, ''but now the future has arrived.'' Berger agrees. ''I think there are clearly gene delivery systems that work,'' he says. ''AAV (adeno-associated virus), I think, is ready for prime time, is already in prime time. The challenge now is to charge forward with clinical trials.''

Once ARIAD successfully regulates the transcription of one gene, others should follow rapidly. But the first project to enter the clinic involves the regulation of signaling proteins. AP1903, ARIAD's 'bumped' homodimerizing drug based on the immunosuppressant FK506, has passed a phase I trial. In the current phase II trial, AP1903 will be used to induce the death of infused T cells if the cells initiate an unwanted graft-versus-host response in leukemia patients undergoing bone-marrow transplants. The T cells are infected with a retrovirus ex vivo, selected for the presence of the transfected gene based on a cell surface marker, then injected into the recipient. AP1903 dimerizes the cell death molecule Fas, based on an added FKBP (FK506 binding protein) domain.

In a variation on the death program, your favorite stem cell can be expanded using a dimerized growth receptor. Although the program is just beginning, ''it's turned into an embarrassment of riches,'' says Clackson. Hepatocytes may be the first test case.

ARIAD plans to activate transcription of therapeutic genes using variants of the heterodimerizing immunosuppressant rapamycin, which binds both FKBP and FRAP proteins. The system has been tested successfully in monkeys, as reported in *Science* in January 1999. The first candidate protein for this approach will be Epo, because it is an important therapeutic whose gene patent happens to expire in 2004. Those genes that do not have expiring patents will have to be licensed, on a gene-by-gene basis. Berger plans to drive a hard bargain. ''This is not going to be given out for a few percent royalty,'' he says. ''You don't have a product without our technology. We have the key, differentiating technology.''

In the research market, dimerizing reagents have already been distributed to nearly 400 academic laboratories in exchange for future intellectual property rights, and marketing to biotech companies for use in research begins January 2001.

The Tet alternative

In the research market, ARIAD faces stiff competition from the tetracycline-induced systems developed by Hermann Bujard (University of Heidelberg, Germany). According to Bujard ''Tet is by far the most broadly used and applied'' transcriptional regulatory system, although Clackson emphasizes that the ARIAD system can also be used to regulate signal transduction proteins directly.

Bujard deliberately chose tetracycline, he says, ''because with Tet you enter a whole field of known pharmacology and chemistry.'' The disadvantage, though, is that the tetracycline-responsive transcription regulators are bacterial, not human proteins. ''The real concern for gene therapy is determining whether (the system) is by itself immunogenic,'' says Bujard. Based on mouse experiments, he says, things look good.

Right now a more pressing concern is the lack of organizational impetus. Bujard is trying to buy back the rights to his technology from BASF. ''For such a huge company this was not an important enough asset to further develop,'' he says. ''They have decided they want to sell it, and I think it should be put in a small company.''

However those negotiations play out, Bujard is happy with his system. ''If you look at its impact in creating especially conditional mutants in mice it has been just fantastic,'' he says. ''Its main impact will be target validation. In gene therapy it may work; it may not work.''

Yet another alternative was presented in the November 23 *Nature* by Ji-Won Yoon (Yonsei University, Seoul, Korea), who cured diabetic mice with an insulin gene hooked up to a glucose-responsive promoter. Regulated

promoters don't require pills, but they are not generalizable like ARIAD's system.

Expression on demand

When expression just can't wait, ARIAD has the RAPID system, in which a drug frees pre-made protein from an aggregate, so it can be secreted promptly into the bloodstream.

"We had devised a (rapid delivery) scheme," says Clackson, "but the key thing we needed was a conditional aggregation domain (CAD). By pure chance we discovered such a protein. It sat on the shelf for a while until we realized that it would work with RAPID."

The CAD (now called F_M) was one of the FKBPs engineered with a hole to accommodate AP1903. F_M was the one variant that, unexpectedly, gave a positive two hybrid when paired with itself. The aggregation was dissociated by a monomeric version of AP1903.

Several F_M domains strung together cause a protein to aggregate in the endoplasmic reticulum unless the monomer drug is added. Protein (e.g. insulin) release can be detected by 15 min after drug addition, and peaks within 2 h. The system is particularly well suited to proteins that must be pulsed rapidly to be effective, such as parathyroid hormone for osteoporosis or beta-endorphins for chronic pain.

RAPID is far from the clinic, but has generated a *Science* paper. So is science triumphing over business? Clackson thinks not. "We've got a more ruthless product focus than we've had in the past," he says.

And Berger has no regrets about the good science. "The challenges have been scientific," he says. "These have been hard programs to pursue. If you overcome these challenges you create something of great value, and I think we are there now."