

Oligo oligarchy—the surprisingly small world of aptamers

Has skepticism about nucleic acid therapeutics shaped the competitive landscape for emerging aptamer companies?

Karl Thiel reports.

After a hiatus of over two years, the biotech initial public offering window is open—but with a different focus. Where investors once threw cash at companies with platform technologies but no products or foreseeable prospects of profits, now they want late-stage products addressing large audiences and unmet medical needs. But amid this conservative focus, there has been an appetite for something a little racier—aptamers, oligonucleotide-based therapeutics that directly bind proteins. Three of 21 companies that go public since last October—Eyetechn (New York), Dynavax (Berkeley, CA, USA) and Corgentech (S. San Francisco, CA, USA)—work on this largely unproven modality. A fourth company, Archemix (Cambridge, MA, USA), raised \$50 million in April—an IPO-like sum in this market. All this, despite the fact that these drugs are chemically similar to two largely failed modalities of the past, antisense and ribozymes.

Don't expect a slew more aptamer companies to earn their tickers anytime soon, however. As remarkable as is the interest in this therapeutic strategy is the concentration of intellectual property (IP) assets into just a few players that appear to have successfully blocked most competitive threats. (See Table 1 for a list of companies.)

Third time the charm?

Aptamers are relatively short (usually 12–30 base) single-stranded oligonucleotides that assume specific, stable conformations *in vivo* and bind tightly to very specific protein targets. In terms of chemical composition, these nucleic acid ligands are essentially identical to antisense drugs—indeed, RNA aptamers have been modified in some of the same ways as antisense molecules to avoid problems of rapid nuclease degradation in the bloodstream and short serum half-life. But because they target proteins, aptamers, unlike antisense drugs, can work against either intra- or extracellular targets—obviating the need, at least for some applications, to get the molecules inside of cells.

Yet whereas antisense and another nucleic acid-based therapeutic technology, ribozymes, have brought investors more heartache than success, the reception for aptamers remains enthusiastic. (The most recent setback for antisense came on May 3, when an FDA panel rejected Genasense, an antisense drug for malignant melanoma developed by Genta (Berkeley Heights, NJ, USA) and Aventis (Strasbourg, France), that produced equivocal clinical results.)

How did aptamers, an even younger nucleic acid-based modality, gain the confidence of twice-shy investors? The ambassador for this new kind of therapeutic is a drug called Macugen (pegaptanib sodium), being developed by Eyetechn for the treatment of the 'wet' forms of age-related macular degeneration (AMD). Although many companies that became newly public in 2003 brought disappointing returns to investors, Eyetechn brought in a one-day return of 54% and has been the ninth-best-performing IPO in the past 12 months across all industries. Despite the fact that Macugen is Eyetechn's only product—there is nothing else in the company's pipeline even remotely close to the clinic—Eyetechn commands a market cap north of \$1.4 billion.

That rich valuation can be attributed to some very robust results in interim analyses of two pivotal phase 2/3 trials, and to the huge potential market for Macugen—some 1.6 million cases of wet AMD in the United States alone, with an estimated 200,000 new cases each year, a figure likely to grow as our population ages. A sweetheart deal the company landed with Pfizer (Groton, CT, USA) didn't hurt either—it brings Eyetechn up to \$750 million in various payments and still leaves the young company an equal share of all profits. Eyetechn intends to submit a licensing application to the FDA in the third quarter of this year, and with an accelerated approval agreement from the agency, Macugen stands a very good chance of reaching the public in early 2005.

Class distinction

What is more exciting to some venture capitalists than this single product, however, is the

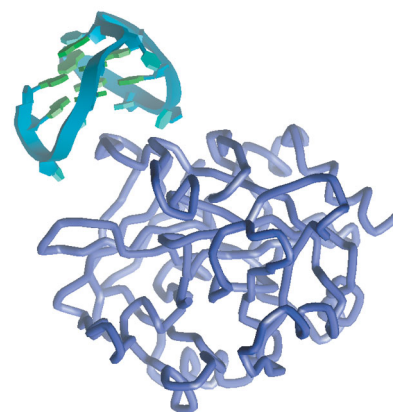


Figure 1 The thrombin aptamer (aqua) forms a specific binding surface with the thrombin protein (blue).

virtues of aptamers as a therapeutic class to treat any number of illnesses. In theory, these molecules offer some of the best of both small molecules and monoclonal antibodies.

On the small-molecule side of the equation is the stability and seeming lack of immunogenicity of aptamers. Anthony Adamis, Eyetechn's CSO and cofounder, describes them as "rock stable."

"You can heat them up to 80 or 90 degrees [Celsius], cool them down, and they will still work," he says. "They can survive in rather harsh environments with various solvents." Adamis believes the natural stability of aptamers will eventually help his company develop an extended release or alternative delivery formulation of Macugen, which was given by intravitreal injection every six weeks in clinical trials. In addition, no evidence of antibody development or other immunogenicity to Macugen was observed in clinical trials, he says—a claim echoed by others who have used aptamer therapeutics in the clinic.

Aptamers may also share some common ground with small molecules when it comes to manufacturing. Making Macugen, says Adamis, "is a straightforward 15-step synthetic process. Unlike an antibody, where you have to build a gigantic fermentation plant, this is straightforward chemical synthesis." Archemix president and CEO Errol De Souza adds that the synthetic process is also relatively free of patent and royalty entanglements that surround antibody production.

On the antibody side of the equation is the specificity and binding affinity of aptamers. Aptamers have a larger surface area in comparison to small molecules, offering more topological and chemical points of interaction with targets, and thus antibody-

like dissociation constants (Fig. 1). In terms of specificity, Adamis notes, “aptamers have been created that have a 10,000-fold greater affinity for caffeine versus theophylline—and these are molecules that differ only by a methyl group.” Macugen, for instance, binds to only one of six isoforms (isoform 165) of vascular endothelial growth factor (VEGF), a specificity that Adamis argues makes the drug safer and more effective than a complete VEGF blockade.

Aptamer oligopoly

With these traits in mind, some investors are betting that aptamers will find a significant niche in tomorrow’s pharmacopeia. And unlike antibodies, where entrepreneurs must brave a very competitive landscape, the intellectual property (IP) rights surrounding aptamers are remarkably centralized.

That concentration can be attributed in large part to Larry Gold, one of the developers of an *in vivo* process for selecting aptamers out of large combinatorial pools of oligonucleotides, called SELEX¹ (for systematic evolution of ligands by exponential enrichment). Gold, along with venture capitalist Patrick Mahaffy, founded NeXagen (Boulder, CO, USA) in 1991 to exploit this technology.

The company later merged with Vestar to become NeXstar, which was in turn acquired by Gilead Sciences (Foster City, CA, USA) in 1999.

Although a decade of work went into aptamers, including the development of Macugen (then called NX-1838) as far as a phase 1 trial, Gilead’s management decided to focus the business around the company’s antiviral drug franchises, and to divest both the aptamer and oncology assets of the company. Gold has since gone on to found SomaLogic (Boulder, CO, USA), which is focused on diagnostic applications of aptamers.

Eyeteck licensed exclusive rights to Macugen from Gilead in 2000—indeed, the company was formed specifically to take advantage of that licensing opportunity. In October 2001, the rest of Gilead’s aptamer assets went to Archemix, with Gilead receiving \$17.5 million plus warrants in Archemix.

Using the SELEX process, to which Archemix now owns the exclusive rights for discovering therapeutic molecules, researchers are able to screen huge libraries on the order of 10^{15} – 10^{16} unique oligonucleotides against their targets. The SELEX estate includes not just this discovery process, however, but over 175 patents on specific com-

positions and methods of use—including claims as broad as “a non-naturally occurring nucleic acid ligand having a specific binding affinity for a target molecule, said target molecule being a protein, wherein said nucleic acid ligand is not a nucleic acid having the known physiological function of being bound by the target molecule”². In other words, they have rights to any nucleic acid sequence that binds to any protein—the entire universe of synthetic aptamers—as long as it doesn’t involve a known natural process, such as the interaction between transcription factors and DNA.

One key way in which aptamers differ from both small molecules and antibodies is in the potential universe of drug candidates. With potential therapeutics limited to oligonucleotides ranging from about 10 to 40 bases in length, there is a finite number of molecules to be screened—a stark contrast to combinatorial synthesis of small molecule libraries, where even the largest collection represents an infinitesimal piece of conceivable chemical space.

The three-dimensional diversity of aptamers is somewhat smaller than potential sequence diversity, suggests Archemix executive vice president Martin Stanton. Reversing

Table 1 Therapeutic aptamers in clinical development

Company (partner)	Drug (indication)	Status
Aptamera (Louisville, KY, USA)	Agro100 (cancer)	Phase 1 launched September 2003
Archemix (Nuvelo) (Lyon, France)	ARC-183 (short half-life anticoagulant/antithrombotic for CABG)	Phase 1 planned for second half of 2004
Coley Pharmaceutical Group (Wellesley, MA, USA)	ProMune (non-small-cell lung cancer, melanoma, cutaneous T-cell lymphoma)	Phase 2; phase 3 trial planned for late 2004. Phase 1/2 data available late 2004; phase 2 planned for second half 2004
(GlaxoSmithKline and Chiron) (Aventis)	VaxImmune adjuvant (cancer) Asthma and allergy compounds	Phase 1—development done by partners Two products scheduled to enter phase 1 in 2004
Corgentech (S. San Francisco, CA, USA) (Bristol-Myers Squibb)	E2F decoy (to prevent vein graft failure)	Enrollment complete in two phase 3 trials. Phase 1/2 trial for arteriovenous graft failure planned for first half of 2004.
Dynavax (Berkeley, CA, USA) (UCB Farchim)	AIC-1018 ISS linked to ragweed allergen (ragweed) 1018 ISS as adjuvant mixed with hepatitis B allergen as prophylaxis. (A-linked version for therapeutic use is in preclinical development)	Phase 2/3 trial launched February 2004. Phase 3 trial planned for 2004.
(Berna Biotech)	1018 ISS inhalation (asthma)	Phase 2, results in mid-2004
Eyeteck (Pfizer) (New York, NY, USA)	Macugen (all forms of wet AMD)	Interim results of two pivotal phase 2/3 trials reported; NDA filing planned for third quarter 2004
(Pfizer) (Pfizer)	Macugen (diabetic macular edema) Macugen (retinal vein occlusion)	Phase 2 trials ongoing Phase 2 trial planned for 2004
Hybridon (San Diego, CA, USA)	HYB2055 (cancer)	Phase 2 planned for summer 2004

AMD, age-related macular degeneration; CABG, coronary artery bypass grafting; NDA, new drug application.

a C and G in the structural portion of the molecule, for instance, does not appear to make a difference to the final shape of the molecule. Thus, a library of 10^{16} oligos is a pretty good-sized chunk of the practical aptamer universe. Using SELEX, Archemix researchers are able to get to success or failure more quickly than they might with conventional small molecule screening.

Backdoor into cells

Other interactions between nucleic acid ligands and proteins that have “known physiological function” would hence presumably fall outside of Archemix’s broad net. Three companies—one of them freshly public this year—are working on aptamers that target Toll-like receptor 9 (TLR9) on dendritic cells (Fig. 2). This receptor appears to provide a solution to what is otherwise a drawback of aptamers—they don’t get into cells very well. In fact, it appears that TLR9 evolved specifically to bring certain nucleic acids into intracellular compartments called endosomes.

In the genomes of vertebrates, cytosine and guanine (C and G) seldom occur together in sequence—and when they do, the cytosine is usually methylated. Unmethylated CpG dinucleotides, however (where the p represents a phosphate bond), occur frequently in bacterial genomes. It is now widely believed that humans evolved a mechanism to recognize CpGs as signaling a foreign invader, and TLR9 is the gateway by which this foreign genetic information is taken into cells and used to mount an immune response.

This led researchers at both the University of Iowa (Iowa City, IA, USA) and the University of California-San Diego (UCSD) to explore the idea of using CpG-containing immunostimulatory sequences as therapeutics. The resulting UCSD patents were licensed to Dynavax (Berkeley, CA, USA), whereas Arthur Krieg, who while at the University of Iowa made a key discovery on the immunostimulatory role of CpGs³, went on to found Coley Pharmaceutical Group (Wellesley, MA, USA). Currently a patent dispute rages between the two companies concerning the priority of early inventions in the field, but Dynavax and Coley are actually using the technology to somewhat different ends—Dynavax, with a primary focus on allergy, asthma and hepatitis B, Coley with a focus on cancer vaccines. Hybridon (Cambridge, MA, USA) is also working on aptamers to TLR9, but has developed a compound using synthetic immunostimulatory motifs instead of CpGs, which may put them outside the IP battle surrounding Dynavax and Coley.

Smooth sailing

With the exception of this one patent dispute, the aptamer space is remarkably placid. Because of Archemix’s broad ownership of IP rights in the field, there are relatively few competitors in the space and virtually no legal wrangling. It is probably impossible to tell whether the concentration of IP in one company is in any way slowing the potential development of aptamer drugs, but Archemix has thus far been willing to grant licenses to other companies seeking to develop aptamer drugs. “Licensing is at least a portion of their business model,” says Chris Rusconi, vice president of discovery and development at Regado Biosciences (Morrisville, NC, USA), a young company working on “antidote-controlled” aptamers, where a therapeutic effect can be turned off by introducing a sequence complementary to a portion of the therapeutic sequence. “Do they want to go and enable a thousand little competitors? I don’t think so. But they’ve contemplated this and they’ve been a very good corporate partner.”

Archemix has likewise granted a license to Aptamera (Louisville, KY, USA), which is developing an aptamer that targets nucleolin, a target found on the surface of many kinds of cancer cells. In both these cases, Archemix has kept a stake in any resulting commercial drugs; they have also granted a license to Noxxon Pharma (Berlin) that does not bear royalties. Noxxon is developing ‘Speigelmern,’ L-form mirror-image aptamers that have greater stability *in vivo* than unmodified RNA⁴. Other aptamers in clinical development have achieved stability against nuclease degradation through a bag of tricks used with antisense drugs—2-OH modifications of ribose and 3’ ‘end caps’ to block exonuclease activity, or modifications of backbone linkage or bases.

Overcoming obstacles

There are a few drawbacks to aptamers. In terms of manufacturing cost, they are much more like antibodies than small molecules. Eyetech isn’t revealing manufacturing costs for Macugen, but Archemix’s Stanton estimates that his company’s first clinical candidate, an anticoagulant called ARC-183, will cost \$50 or less per gram at manufacturing scale. Since aptamers have a lower molecular weight than antibodies but similar binding affinity, they should cost far less than antibodies on a per dose basis (1 gram of Macugen yields 3,000 doses, notes Eyetech’s Adamis), but still much more than small molecules.

A more serious limitation of aptamers is familiar to those who have worked with other

nucleic acid therapeutics: they do not, under normal circumstances, get inside of cells. “Aptamers get in as well or as badly as antisense or siRNA,” acknowledges De Souza, but since they target proteins instead of message, drug developers have more flexibility in selecting their targets. “As part of our business strategy, we’ve said in the first phase of our business plan that all of our targets will be extracellular—just because we do not want to struggle with the cell barrier that antisense has struggled with.”

Perhaps the most limiting drawback has to do with serum half-life. Unlike antibodies, which can linger in the bloodstream for days or even weeks, aptamers are quickly eliminated by the kidneys. ARC-183, deliberately designed for a short duration of action, has a half-life of about 2 minutes. This can be increased by attaching polyethylene glycol (PEG) molecules to the oligonucleotides—Macugen, for instance, is linked to two branching 20 kDa PEGs—but there are limits to what this can achieve. The longest half-life Archemix has achieved for any aptamer is just over 24 hours, although Stanton notes that this result is from rats and should double or more in humans. De Souza argues that the ability to “dial up and down” half-life with different size PEGs can be an advantage, but it appears that the flexibility is for now somewhat limited on the upper end.

Nevertheless, if Eyetech gains US marketing approval for Macugen in 2005, it will be a huge vindication for aptamers as a therapeutic class—one that antisense and ribozymes have never really earned—and proof that Eyetech’s founders made a shrewd gamble by licensing Macugen when it was in phase 1. “Eyeteck took a risk on a whole new therapeutic modality,” says De Souza. With the current enthusiasm surrounding this class of molecules, it may no longer be clear how risky that decision seemed a few years ago. Eyeteck’s success will probably benefit Archemix as well. “We founded this company on the belief that aptamers are underappreciated and ready to be commercialized,” says Stanton. “It was a bit of a leap at the time, and I don’t think it was widely appreciated.”

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