Safety signal dampens reception for mipomersen antisense

In February, Isis announced its cholesterol-lowering antisense therapy, mipomersen, had met its endpoints in a phase 3 trial of 124 patients with heterozygous familial hypercholesterolemia (FH) and coronary artery disease. The news ought to have sent share prices soaring, as mipomersen, if approved, could rival blockbuster statin drugs. Instead, lingering concerns related to high liver enzyme levels in some trial participants—a safety signal that had been noted in previous trials—dampened investor enthusiasm. But even if elevated liver enzymes make it less likely that mipomersen will supplant statins in major indications like coronary artery disease, efficacy data for the antisense therapy in patients with homozygous FH remain compelling.

The latest trial results provide validation that antisense drugs do have sufficient potency to rival traditional small-molecule drugs. After the clinical and regulatory disappointments of antisense therapy’s other poster child, Genasense (oblimersen), Alan Gewirtz, an antisense researcher at the University of Pennsylvania School of Medicine, Philadelphia, says the results are a “shot in the arm” for the field. However, given that one touted advantage of antisense compared with other drugs is the specificity of mechanism and potentially reduced off-target effects, the elevated liver enzymes associated with mipomersen therapy are, on the one hand, disappointing. On the other, these observations are potentially informative about idiosyncratic liver toxicity specifically associated with targeting lipid biogenesis and transport.

Mipomersen is one of Isis’s second-generation antisense molecules that is delivered systemically (Nat. Biotechnol. 25, 497–499, 2007). The Carlsbad, California–based company is a trailblazer for oligonucleotide therapy. In 1996, it received approval for Vitravene (fomivirsen), the first antisense treatment approved by the Food and Drug Administration.

Table 1  Antisense oligonucleotides in phase 3 testing

<table>
<thead>
<tr>
<th>Company/partner</th>
<th>Drug</th>
<th>Mechanism</th>
<th>Indication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisense Pharma</td>
<td>Trabedersen</td>
<td>A phosphorothioate antisense oligo targeting a transforming growth factor beta 2 (TGF-β2) and tumor necrosis factor ligand 13</td>
<td>Glioblastoma and anaplastic astrocytoma</td>
</tr>
<tr>
<td>Atlantic Healthcare</td>
<td>Alicaforsen</td>
<td>2‘-O-(2-methoxy) ethyl-modified ribose antisense oligo targeting intercellular adhesion molecule 1</td>
<td>Ulcerative colitis, Crohn’s, inflammatory bowel disease, pouchitis, asthma</td>
</tr>
<tr>
<td>Genesys (Epalinges, Switzerland)</td>
<td>Aganirsen</td>
<td>A 25-mer phosphorothioate antisense oligo targeting insulin receptor substrate-1</td>
<td>Corneal graft rejection</td>
</tr>
<tr>
<td>Genta</td>
<td>Genasense (oblimersen)</td>
<td>A phosphorothioate antisense oligo targeting BCL-2</td>
<td>Melanoma, chronic lymphocytic leukemia and various other blood cancers and solid tumors</td>
</tr>
<tr>
<td>Isis Pharmaceuticals/ Genzyme</td>
<td>Mipomersen</td>
<td>2‘-O-(2-methoxy) ethyl-modified ribose antisense oligo targeting ApoB</td>
<td>FH and hypercholesterolemia</td>
</tr>
<tr>
<td>NovaRx (San Diego)</td>
<td>Lucanix (belagenpumatucel-L)</td>
<td>Cells derived from four non-small cell lung cancer cell lines transfected via electroporation with a plasmid encoding a TGFB-β2 antisense gene</td>
<td>Astrocytoma, lung tumors</td>
</tr>
<tr>
<td>OncoGeneX/Teva</td>
<td>Custirsin</td>
<td>2‘-O-(2-methoxy) ethyl-modified ribose antisense oligo targeting clusterin</td>
<td>Castration-resistant prostate cancer, solid tumors</td>
</tr>
</tbody>
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Source: Thomson Pharma Partnering; IDDB
IN brief

*Bt* brinjal splits Indian cabinet

India’s prime minister, M. Singh, has intervened in the political wrangle that erupted over a genetically modified (GM) eggplant strain due for commercial release. Approval of the locally developed *Bacillus thuringiensis* (Bt) variety appeared imminent, but on February 9, the minister of environment and forests, Jairam Ramesh, responded to public opposition by declaring an indefinite moratorium on the approval of *Bt* brinjal, as it is known locally, on the grounds of insufficient data to confirm that it is safe to eat. This decision has created a cabinet rift, prompting Singh to hold a consultation with senior government officials. *Bt* brinjal is India’s first locally developed GM food crop and was created by Mahyco, a Jalna-based Maharashtra Hybrid Seeds Company in a joint venture with Monsanto, the St. Louis–based seed giant. Last October, the Genetic Engineering Approval Committee (GEAC), India’s official regulatory body for registering GM organisms, approved release of the transgenic brinjal, opening the door for commercialization of another dozen or so GM crops in the pipeline. The environment minister’s decision to over-rule the GEAC was unexpected. But it followed vociferous feedback from civil societies and advice from scientists, including Monkombu Swaminathan of ‘green revolution’ fame and Pushpa Bhargava, founder of the Centre for Cellular and Molecular Biology in Hyderabad, that additional safety testing of the GM brinjal was warranted. Chavali Kameswara Rao, secretary of Bangalore-based Foundation for Biotechnology Awareness and Education, believes the environment minister caved in to intense lobbying pressure from activists. He fears the resultant delay of commercialization will promote clandestine cultivation of *Bt* brinjal—similar to what happened previously in India with *Bt* cotton (*Nat. Biotechnol.* **22**, 1333–1334, 2004). On February 24, Singh and senior cabinet members agreed to set up a national biotech regulatory authority to oversee registration of transgenic products and requested that the GEAC resolve outstanding safety concerns relating to *Bt* brinjal. No time frame for these deliberations was set, however. The Department of Biotechnology (DBT), the main funding agency for transgenic research, has stayed out of the controversy. But as Prasanta Kumar Ghosh, DBT’s former head of biosafety puts it, “There is no scientific basis for a moratorium.” Bhim Sain Bajaj, president of the Hyderabad chapter of All-India Biotech Association, agrees. “It is a big mistake...the time will come when India will have to import rice and vegetables and we will regret [this decision].”

Killugudi Jayaraman

Administration (FDA) for inflammation of the retina in cytomegalovirus-infected patients. Although a breakthrough experimental therapy, the treatment has not been able to build a substantial commercial market.

The company’s current lead antisense product will compete for market share with the statins, a highly successful group of drugs (including Pfizer’s blockbuster Lipitor; atorvastatin) that reduce levels of cholesterol, low-density lipoprotein (LDL), apolipoprotein B100 (ApoB), and triglycerides through inhibition of 3-hydroxy-3-methyl glutaryl co-enzyme A (HMG-CoA) reductase. Instead of targeting HMG-CoA reductase, mipomersen acts directly on ApoB, the protein responsible for carrying plaque-thickening LDL cholesterol into the arteries. As a second-generation 2’-O-(2-methoxy)ethyl-modified ribose oligonucleotide, the DNA oligonucleotide exhibits high affinity for ApoB messenger RNA. Binding of the ApoB mRNA by mipomersen then triggers cellular ribonuclease H to hydrolyze RNA phosphodiester bonds, thereby inhibiting translation and suppressing levels of ApoB protein.

On the basis of previous clinical data published last month (*The Lancet* **375**, 998–1006, 2010), mipomersen seems to do a better job than statins at fighting the dramatic cholesterol levels in individuals with FH, a rare genetic disorder. In the severe form of the disease, which affects 10,000 people worldwide, homozygous patients often have LDL levels up to six times normal, making patients susceptible to heart attacks as early as childhood. Phase 3 studies in homozygous patients led to an impressive 25% drop in LDL levels. This success prompted Isis and its partner Genzyme of Cambridge, Massachusetts, to initiate a phase 3 trial in heterozygous patients, with a view to seeking approval in the 1.5 million people who have less severe forms of FH and then extending the therapy to anyone with high cholesterol that is insufficiently controlled by statins (*Nat. Biotechnol.* **26**, 148, 2008).

The trial results announced in February for the heterozygous FH population included 124 individuals with pre-existing coronary artery disease, who were already taking maximum-tolerated doses of statins. After 26 weeks of treatment, mipomersen achieved a 28% reduction in LDL, compared with a 5% increase among controls. Isis stated that 45% dipped below 100 mg/dl—the recognized treatment goal. The study also met three secondary endpoints, with reductions in ApoB, total cholesterol and non-high-density-lipoprotein cholesterol.

The news was not all positive, however. Elevated liver enzymes were observed in 12 of 83 patients; in 5 patients, levels reached three times the upper limit of normal. It was these results, when made public, that prompted the company’s shares to plummet by 17%. “Alone, [the liver enzyme increase] is not indicative of liver toxicity, but I think it serves as such a red flag, and investors have been burned so many times, they’re not going to mess with this,” says Edward Tenthoff, who is a senior research analyst and managing director at Piper Jaffray in Minneapolis.

In the earlier homozygous FH trial, 28 patients on mipomersen experienced a 24.7% decrease in LDL, compared with a 3.3% reduction among the placebo group. Four patients (12%) had increases in concentrations of the liver enzyme alanine aminotransferase (ALT) of at least three times the upper limits of the normal range. Other liver tests, including levels of bilirubin, albumin and prothrombin, showed no signs of liver damage, according to the company.

Importantly for antisense technology as a whole, the spike in enzyme levels is not entirely unexpected; in fact, it is likely to be target specific, given that lipid-lowering drugs are known to be one of the rare examples in which a pharmacodynamic property of the drug class as a whole accounts for liver toxicity. Statins, for example, are associated with a dose-related increase in the incidence of the liver ALT three times greater than the upper limits of the normal (threshold set by regulators); indeed, acute liver failure occurs in about one in a million statin-treated patients. The 124 patients in the mipomersen trial were already on high doses of statins.

For its part, Isis believes that elevated liver enzymes are not an inherent problem of antisense technology. For one thing, individuals who had the steepest drop in LDL and ApoB levels on mipomersen tended to have higher enzyme levels. What’s more, ~5,000 people have been treated with oligonucleotides against other targets, with no evidence of ALT increases, according to Geary. “[The elevated enzymes] are more likely an on-target side effect that’s related to the mechanism. I don’t know that this is necessarily a black mark on antisense,” says Brian Abrahams, senior biotech analyst at Oppenheimer & Co in New York.

As yet, the mechanism underlying the increase in liver enzymes in a small number of patients is not well understood. Possible explanations include alterations in the lipid concentrations in hepatocyte membranes, which could cause mild hepatic cell dysfunction, “but as far as I can tell it’s uncer-
tain,” says Cy Stein, professor of medicine and molecular pharmacology at the Albert Einstein College of Medicine in New York. By acting on ApoB, a carrier for lipids, mipomersen may also affect fat accumulation in the liver. Suppression of ApoB may lead to the accumulation of fats in cellular lipids, which might trigger the raised liver enzyme levels observed. This idea is supported by some patients with a genetic condition called hypobetalipoproteinemia, who cannot make ApoB and have low levels of LDL. Some of these patients accumulate liver fat. Thus, mipomersen could, in effect, be mimicking that condition, says Robert Hegele, a professor of medicine and biochemistry and director of the Blackburn Cardiovascular Genetics Laboratory at Robarts Research Institute in London, Ontario. Still, “It’s all speculation [at this point],” he hastens to add.

Antisense therapies as a class, on the other hand, do have off-target effects of their own. One issue is that the highly charged phosphorothioate backbone binds tightly to charged residues in proteins—a property that helps them avoid elimination via the kidney through association with albumin proteins. But this property might also lead to the binding of antisense to proteins on the surface of hepatocytes, perhaps mimicking hepatic and leading to abnormalities, according to Stein.

Despite the recent setback, Genzyme management, which is partnered with Isis on mipomersen, remains sanguine, noting that the effects on liver enzymes were reversible. “Physicians can manage (side effects) by backing off the medication because they’re seeing such a significant drop in LDL,” says Paula Soteropoulos, vice president and general manager of Genzyme’s cardiovascular business. But some analysts think it’s likely that mipomersen will be approved only for homozygous FH patients, who cannot metabolize LDL due to a lack of functional LDL receptors responsible for clearing LDL from plasma. Even if it were approved for a broader population, mipomersen might not have sufficient advantages to convince physicians to switch from small-molecule statin therapies that are administered orally rather than subcutaneously injected.

In any case, Genzyme plans to file in the first half of 2011 in the US and Europe, targeting patients with homozygous FH and possibly severe hypercholesterolemia. Both indications together represent ~25,000 patients in the US and Europe.

The impressive efficacy of mipomersen is testament to Isis’s investment and optimization of second-generation antisense technology. These second-generation chemistries improve stability and binding, Soteropoulos says, and Isis has spun off or licensed the technology to other companies, such as OncoGeneX, located in Bothell, Washington (Table 1), and Altair Therapeutics, of San Diego.

Not everyone is convinced that a rejuvenation in antisense approaches is on the horizon, however. “Isis is the only company left. Other companies have converted to CpG or siRNA [small interfering RNA] approaches,” says John Rossi, a professor of molecular and cellular biology at the City of Hope’s Beckman Research Institute, Duarte, California. The main advantage of RNA interference (RNAi) over antisense has been its greater potency. “RNAi is long lasting. Once it’s engaged the RNA silencing complex, [siRNA] can last for weeks,” says Rossi. University of Pennsylvania researcher Gewirtz, agrees: “It just seems easier to find an RNA molecule that gets you into the game than it is to find an oligo. That’s why RNAi became so widely accepted—it just works for everybody.”

Even so, siRNA and CpG suffer from the same issues of off-target effects and delivery as antisense, and the latter has other advantages. Its easier to manufacture than siRNA, and because antisense has been around longer, there is more clinical experience behind it. There have been 20 or so clinical studies involving antisense, according to Tenthoff, whereas RNAi trials are still in the single digits. “I don’t think antisense is yet giving way to RNAi. Antisense is still a more clinically experienced technology,” says Tenthoff.

“I’m very enthusiastic about both approaches,” says Raymond P. Warrell Jr., CEO of Genta. “The advantage of antisense is that there are now 15-plus years of clinical experience with it. The folks working primarily on RNAi are in the process of relearning a lot of [those] lessons.” He expects the two technologies to ultimately be complementary. “Whether you use RNA or DNA depends to some extent on the target and to some extent the technique you have experience with. The challenge remains first and foremost to identify a critical target so you have a high level of confidence that knocking it out or down will have a transformative effect on the disease,” says Warrell.

The number of drug approvals for orphan indications has doubled in recent years, according to a report from the Tufts Center for the Study of Drug Development. The independent, nonprofit research group at Tufts University in Boston, found that between 2000 and 2002 the US Food and Drug Administration (FDA) approved 208 orphan drugs, and the number climbed to 425 between 2006 and 2008. The increase could reflect the fact that orphan diseases are simple to target as they are often underpinned by a single genetic cause. But financial incentives for pursuing orphan drugs, such as a waiver of the FDA’s $1.4 million filing fee, long marketing exclusivity and high prices charged are likely factors, too. According to FDA data, biotech firms generate 50% of orphan drug applications and academia another 25%. Pharma makes up less than 25% of the total probably because “ orphan drugs do not regularly fit their business model,” says Tim Coté, director of the FDA’s Office of Orphan Product Development. That has been changing, however. Regeneron, of Tarrytown, New York, has seen this firsthand with Arcalyst (rilonesat), an interleukin-1 (IL-1) ‘trap’ to treat cryopyrin-associated periodic syndromes (CAPS), a rare disease that affects only a few thousand people globally. In 2003, Novartis of Basel terminated a collaboration with Regeneron over the IL-1 trap, because the Swiss pharma was not interested in the small market for CAPS. Later, Novartis developed an IL-1 antibody for CAPS, Ilaris (canakinumab), which gained approval in July 2009.

Pharma’s Asian syndicate

Three big pharma—Pfizer, Merck, and Eli Lilly—are pooling their resources to set up an independent nonprofit company to spur research into innovative treatments for cancers common in Asian populations. The new Asian Cancer Research Group (ACRG) will build an open-access pharmacogenomic cancer database, which will be made publicly available to researchers in the field. Wu Jun, vice president of Xiangxue Pharmaceutical, Guangzhou, says, “It will save Western companies time and money and is good news for patients in China.” The joint venture will focus initially on lung and gastric cancers and aims to gather 2,000 tissue samples over the next two years. “ACRG could get more data from Asia and spend less on research compared with what they spend in the West,” says Wu. ACRG is an example of a growing trend in pre-competitive collaborations. The same three companies have done it before with Enlight Biosciences (Nat. Biotechnol. 26, 960–961, 2008), an R&D startup for developing drug discovery tools. The pharma giants are searching for ways to capture the emerging Asian markets. Plans for ACRG were already underway before last year’s decision by the Chinese government to invest 850 billion yuan ($125 billion) on healthcare reform, according to a spokesperson for Merck.