RNA-bound: the future of antisense drugs

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The use of nucleic acid therapies to block RNA function was first described in 1978. The latest generation of these compounds, LNA antagonists, is now overcoming the main obstacle to the success of its predecessors, through a greater ability to bind to RNA.

Innovative approaches to drug discovery and disease treatment are the basis of the biotechnology industry. However, it is a rare event when a new technological approach leaps straight from the lab bench into the market. Invariably, by a process of iterative refinement, a concept evolves through multiple generations before a marketable product is created. A prime and well-recognised example is monoclonal antibody technology, which is in its third and fourth generations. There are now over 100 potential antibody products in clinical development.

A similar evolution is emerging with the development of nucleic acid therapies. The use of a string of DNA nucleotides to bind and block messenger RNA function was first reported in 1978 and, since then, many companies have applied the antisense principle to make oligonucleotide drugs with the aim of switching off the expression of a specific disease-associated protein – the drug blocking or destroying the messenger RNA responsible for the protein's synthesis. The first such drug to be marketed was Isis Pharmaceuticals' Vitravene, a topical treatment for human CMV (cytomegalovirus) retinitis, which was approved by the European Medicines Agency (EMEA) in 1999 and the US Food and Drug Administration (FDA) in 1998. Through a series of chemical improvements, antisense drugs have been refined substantially since then and breakthroughs have been made by a number of companies.

In particular, the discovery of RNA interference – a natural antisense mechanism in plants and animals – has led to the emergence of companies such as Alnylam Pharmaceuticals and Sirna Therapeutics, who are developing synthetic double-stranded RNA as high-potency antisense drugs known as short interfering RNA (siRNA). More recently, the recognition that siRNAs are unwound within the cell by a mechanism known as RNA-induced silencing complex (RISC), and that only one of their two RNA chains (the antisense strand) binds and inactivates the target mRNA, has swung the pendulum back to the potential potency of RNA analogues, such as locked nucleic acid (LNA) as single-stranded high-affinity antisense drugs. The latest generation of nucleic acid therapeutics in the clinic therefore encompasses both double (siRNA) and single (LNA) oligonucleotide compounds.

First generation of RNA inhibitors

The first generation of antisense compounds were made from synthetic DNA monomers, modified only in so far as they contain a sulphur substitution in place of oxygen in the phosphate linkages between nucleotides. This so-called phosphorothioate modification has been used in most clinical oligonucleotides to date, since it goes some way towards enhancing the stability of the drug in the presence of tissue nucleases, and also improves plasma half-life. Both Vitravene and the Genta drug, Genasense – currently the subject of a re-submitted NDA – are DNA phosphorothioates. The main problem for such drugs is their relative

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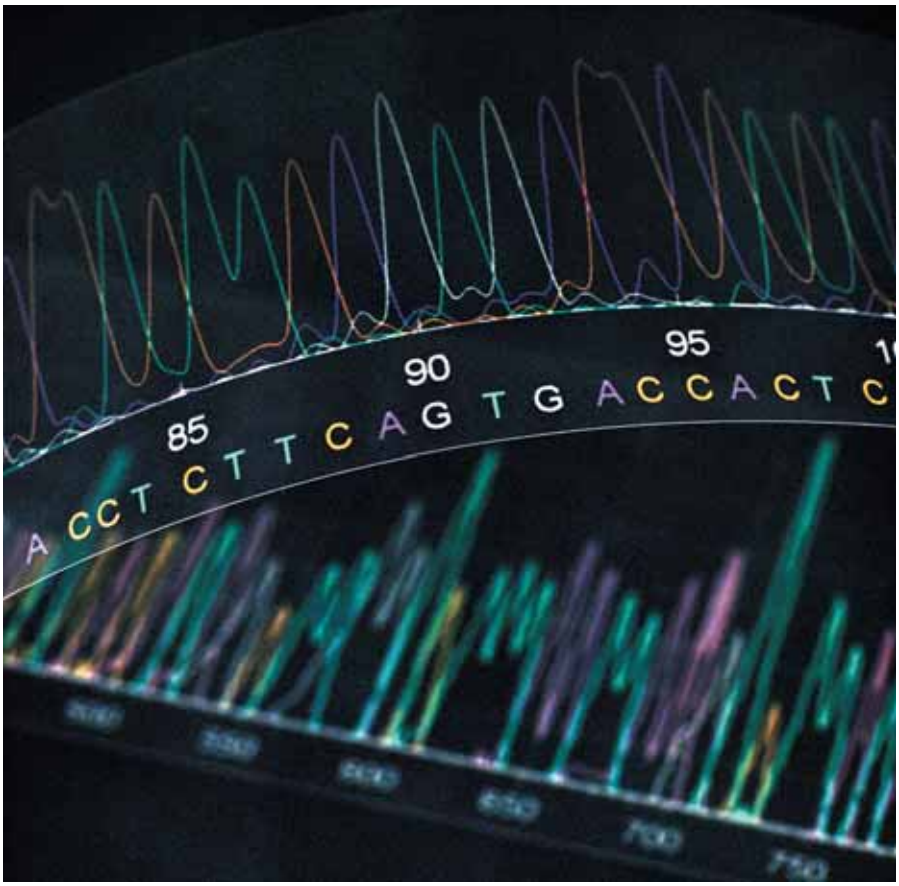

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lack of potency because of weak binding affinity to their target RNA and continuing inadequate resistance to nuclease digestion. Acute toxicities of DNA phosphorothioates reported in primates have also limited the doses at which such drugs can be administered systemically to human patients.

Most experts in the field agree that the first-generation antisense drugs are simply not potent enough to achieve statistically robust efficacy. To date, phase III trials of six separate DNA phosphorothioates have failed to meet their primary endpoint. Only the seventh – Genta's Genasense in a chronic lymphocytic leukaemia (CLL) phase III trial – met its primary endpoint and the drug is being reviewed by US regulators. In the face of such poor results, many companies have sought to develop improved products.

Thus evolved the second generation of antisense compounds, also pioneered by Isis, which acquired a licence for Novartis' 2'-O-methoxyethyl (2'MOE) chemistry. Oligonucleotides consisting wholly or partially of 2'MOE-derivatised monomers have increased resistance to plasma and tissue breakdown. Isis is applying this technology in several areas, including diabetes and cardiovascular disease, and has sub-licensed development of further 2'MOE drugs to Oncogenex and Antisense Therapeutics. However, although they have improved stability over DNA oligonucleotides, 2'MOE compounds show only marginal improvements in the affinity with which they bind RNA.

Other second-generation modifications, such as 2'OMe (pioneered by Hybridon, now Idera Pharmaceuticals) or morpholino-compounds, developed by AVI BioPharma, appear to have no greater potency or benefit than 2'MOE. This lack of potency, particularly when phosphorothioated, is likely to restrict the use of the second-generation antisense compounds to diseases of the liver or kidney, where they achieve relatively high tissue concentrations.

Enter the third generation

There is current excitement in the RNA inhibition field because it is now possible to synthesise compounds with two-to-three orders of magnitude greater RNA-binding affinity. These third-generation antisense drugs fall into two categories: double-stranded siRNA and single-stranded LNA oligonucleotides. In the presence of transfection reagents in cell cultures, both of these third-generation compounds produce significant reductions in target mRNA and proteins at concentrations below one nanomolar. This is dramatically higher than any previous antisense

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OVERVIEW OF OLIGONUCLEOTIDE COMPOUNDS IN DEVELOPMENT IN 2005

Name of compound	Target	Indication	Phase	Nucleotide chemistry	Company
First generation antisense: DNA phosphorothioates (low metabolic stability, low potency)					
Vitravene	CMV	CMV retinitis	Approved	DNA	ISIS
Genasense	Bcl-2	Malignant melanoma	III (NDA withdrawn)	DNA	Genta
	Bcl-2	Chronic lymphocytic leukaemia	III	DNA	Genta
Alicaforsen/2302	ICAM-1	Ulcerative colitis	II	DNA	ISIS
GTI 2040	RNR R2	Renal cell carcinoma	II	DNA	Lorus Therapeutics
GTI 2501	RNR R1	Prostate cancer	II	DNA	Lorus Therapeutics
Second generation antisense: 2'-O-methoxyethyl phosphorothioates (high metabolic stability, moderate potency)					
ISIS 113715	PTP-1B	Diabetes	II	MOE	ISIS
ISIS 301012	ApoB-100	Cardiovascular	II	MOE	ISIS
ATL-1102	VLA-4	Multiple sclerosis	II	MOE	Antisense Therapeutics – licence from ISIS
ATL-1101	IGF-1R	Psoriasis	I	MOE	Antisense Therapeutics – licence from ISIS
OGX-011	Clusterin	Prostate/breast/ non-small-cell lung cancer	II	MOE	Oncogenex – licence from ISIS
LY 2181308	Survivin	Cancer	I	MOE	Lilly – licence from ISIS
Other second generation antisense: 2'-O-methyl, and morpholino analogues (high metabolic stability, moderate potency)					
AEG35156	XIAP	Local and metastatic solid tumours	I	2'OMe	Aegera – licence from Hybridon
Resten-NG	C-myc	Cardiovascular restenosis	I/II	Morpholino	AVI Biopharma
Resten-MP	C-myc	Cardiovascular restenosis	I/II	Morpholino	AVI Biopharma
AVi-4020	West Nile virus	West Nile virus	I	Morpholino	AVI Biopharma
AVI-4126	C-myc	Bladder cancer	I	Morpholino	AVI Biopharma
PD3	Multitargeted?	Chronic obstructive pulmonary disease (COPD)	Preclinical	FANA	Topigen
Third generation – double stranded: siRNA (low metabolic stability, very high potency)					
Cand5	VEGF	Wet AMD	II	siRNA	Acuity Pharmaceuticals
		Diabetic retinopathy	Preclinical	siRNA	Acuity Pharmaceuticals
		Diabetic retinopathy	Preclinical		

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LNA oligonucleotides bind to RNA with extraordinarily high affinity

technology and predicts enormous clinical potential, provided the drugs can get to their site of action in vivo.

Unfortunately, unmodified siRNAs are inherently unstable in the body, with the duplexes unwinding and being degraded by nucleases in the circulation. This may be less of a problem for topical applications and, indeed, Acuity Pharmaceuticals has the most advanced siRNA clinical programme with Cand5 – a local ophthalmic drug to treat wet age-related macular degeneration (AMD), which is in phase II. The product is designed to silence the vascular endothelial growth factor (VEGF) genes that promote the retinal neovascularisation that leads to loss of vision in AMD. Similarly, Alnylam's siRNA drug ALN-RSV01, currently in phase I studies for the treatment of respiratory syncytial virus (RSV) infection, is delivered directly into the lungs.

By contrast, the RNA analogue, LNA, when coupled by phosphorothioate linkages, is remarkably resistant to nuclease cleavage and has a prolonged tissue half-life. In addition, LNA oligonucleotides bind to RNA with extraordinarily high affinity. These characteristics are critical, and result in substantial increases in potency in vivo compared to first- or second-generation oligonucleotides. Santaris Pharma reported data at the American Society of Hematology meeting last December showing that an LNA drug directed against HIF-1a (hypoxia-inducible factor 1) mRNA appeared significantly more effective in reducing tissue hypoxia and VEGF protein levels after systemic administration to mice than the best siRNA directed against the same gene – the comparison was made using the siRNA against HIF-1a described by Yu et al (*Lab Invest* 2004). HIF-1 is a transcription factor that functions as a key regulator of VEGF and VEGF receptor expression and is therefore important in tumour angiogenesis. Additionally, HIF-1a also plays important roles in other cancer processes, such as cell proliferation, apoptosis and cell invasion.

A daunting technical hurdle for

Name of compound	Target	Indication	Phase	Nucleotide chemistry	Company
Sirna-027	VEGF	Age-related macular degeneration (AMD)	I	siRNA	Sirna Therapeutics/partnered with Allergan
	IL4/IL4R	Asthma	Preclinical		
	IL13/IL13R	Asthma	Preclinical		Partnered with Lilly
	VEGF	Solid tumours	Preclinical		
	HBV/HCV	Hepatitis	Preclinical		
ALN-RSV0	RSV	Respiratory syncytial virus (RSV) infection	I	siRNA	Alnylam
NN	HVC	Hepatitis C virus	Preclinical		Benitec
NN	HIV	AIDS lymphoma	Preclinical		
Third generation – single stranded: LNA-based RNA antagonists – LNA phosphorothioates (high metabolic stability, very high potency)					
SPC2996	Bcl-2	Chronic leukaemia	I/II	LNA	Santaris Pharma
SPC2968	HIF-1α	Renal cancer & myeloma	Preclinical	LNA	Santaris Pharma
SPC3042	Survivin	Chemotherapy in cancer	Preclinical	LNA	Santaris Pharma

The evolution of nucleic acid therapies has much in common with the antibody market

siRNA is uptake into cells in the body. SiRNAs are large double-stranded molecules which do not pass readily across cell membranes. Alnylam reported in 2004 that liver uptake, at least, could be enhanced by conjugation of the siRNA with cholesterol. Santaris has matched that by data, presented at a recent Keystone science conference, showing that unconjugated LNA oligonucleotides directed against ApoB100 in the liver are effective in reducing ApoB synthesis and plasma cholesterol levels in mice at doses eight times lower than those required by Alnylam's cholesterol-conjugated siRNA, synthesised as described by Soutschek et al (*Nature* 2004). LNA may transform the opportunity for oligonucleotides as drugs. The much higher binding constants of LNA to complementary RNA sequences, compared to conventional DNA analogues, is such that LNA oligonucleotides can be considered a new class of drug. Santaris has coined the term 'RNA antagonists' to describe such drugs in recognition of their high-affinity binding and target specificity.

Nucleic acid therapies the future

As is clearly demonstrated in the field of antibodies, the early murine antibodies showed great promise, and some progressed onto the market. However, a rapid evolution of the technology followed. Murine antibodies soon became chimeric (part mouse, part human). The second generation of antibodies were described as humanised – in effect recombinant mouse antibodies tweaked to closely resemble human antibodies. The third generation of antibodies were fully human, and now we have further developments into antibody fragments, domain antibodies and nanobodies.

In addition to the 100 in development, this class of compounds now has 20 products on the market.

The evolution of nucleic acid therapies has much in common with the antibody market. Few of the early generation of drugs reached the market, but these are being closely followed by the next generations. Third-generation products based on LNA and siRNA are now in the clinic. As these progress, much will be learned and, before too long, we are likely to see a burgeoning series of more effective nucleic acid-based targeted therapeutics hit the market.

LNA-based RNA antagonists

Locked nucleic acid (LNA) drugs appear to be well tolerated in animals. Santaris has completed GLP (good laboratory practice) toxicology studies with three separate LNA compounds and has observed no clinical, haematological or pathological adverse affects in either rodents or monkeys at clinically relevant doses. Santaris' lead product, SPC2996, is being developed to treat chronic lymphocytic leukaemia (CLL) – the second most common type of cancer of the blood, characterised by a progressive accumulation of long-lived, functionally incompetent lymphocytes.

SPC2996 acts by inhibiting the synthesis of Bcl-2, a key sensor protein that protects cells against apoptosis. The protein is expressed in most cancers but is especially high in CLL where the level of over-expression also correlates with poor outcome. In primate pharmacology studies, SPC2996 has been shown to effectively down-regulate Bcl-2 mRNA and protein when injected intravenously at low doses. The compound is currently being evaluated in an international phase I/II multicentre clinical study at haematology centres in Denmark, France, the UK and the US.

Santaris has two further preclinical candidates in development. The first, SPC2968, was selected from a small library of LNA-based RNA antagonists of HIF-1a mRNA. The other compound, SPC3042, is a potent LNA-based RNA antagonist of survivin mRNA. The latter plays a vital regulatory role in apoptosis, by inhibiting activation of lethal caspases.

In addition, survivin plays a pivotal role in normal mitotic progression and cell division. Survivin is over-expressed in many cancers and in endothelial cells engaged in angiogenesis. However, it is almost absent in normal adult differentiated tissue, thus making it a prime target for cancer therapy.