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Silence is golden: can RNA interference therapeutics deliver?

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Hypes come and go in the biopharmaceutical industry, particularly when it comes to anything that might fit the description of a 'magic bullet'. Immunotoxins, gene therapies, antisense agents and ribozymes have all been hailed as the 'next generation of therapeutics', but, after years of intensive research effort, each has so far failed to fulfil its initial promise. This is doubly frustrating with the advent of the human genome project and the opening up of knowledge of a vast selection of novel genes linked to human disease that are ripe for targeting.

So, what has the current flavour of the month, RNA interference (RNAi), or gene silencing, got to offer, and what makes it different from previous gene-based technologies? RNAi works by blocking expression of specific proteins within the cell at the mRNA level, using short nucleic acid fragments, which initially appears a remarkably similar strategy to those tried-and-tested warhorses of biotechnology – antisense and ribozyme technologies. After years of extensive preclinical and clinical study, neither antisense nor ribozyme strategies have met with significant commercial success, being plagued by delivery problems, inefficiency and doubts about precise mechanisms of action.

The main advantage of RNAi over these techniques is that it appears to be a much more robust and efficient technology. In addition, it is based on a natural process, which could work in favour of clinical success and public acceptance.

Serendipity

RNAi was discovered relatively recently and, in grand Hollywood tradition, was stumbled on almost by accident. In the late 1980s, Richard Jorgensen and researchers at the University of Arizona (USA), while working on transgenic plants, noted an unusual effect when two separate transgenes interacted – gene expression was inhibited rather than stimulated. The effect was particularly striking in a study aimed at deepening flower colour in petunias. The introduction of a gene encoding a pigment-producing enzyme – chalcone synthase – resulted in the flowers actually losing their colour, with them becoming variegated or white.

Elucidation of the mechanism responsible for this effect had to wait until 1998, when a landmark study was conducted by Andrew Fire and colleagues at Stanford University (USA).

Petunia flower exhibiting sense cosuppression (RNAi) patterns of chalcone synthase silencing. Image kindly supplied by Richard Jorgensen.

Instead of plants, they were investigating gene-silencing in the nematode *Caenorhabditis elegans,* and discovered that short stretches of double-stranded RNA were capable of sequence-specific blockage of gene expression. Silencing was later demonstrated in a wide variety of species, including mammals, in which it is presumed to have evolved as a mechanism of protection against viral RNA.

Silencing targets

The mechanism of RNAi, although more sophisticated than conventional direct hybridization (antisense) or RNA cleavage (ribozyme) strategies, is relatively simple. Short double-stranded RNA fragments (around 22 nucleotides long) are the key agents, with the most commonly used type being known as small interfering RNA (siRNA). In nature, these fragments are produced from longer strands by an enzyme known as Dicer. However, in the laboratory, they are typically produced either synthetically or from a vector.

siRNAs contain a sequence that is complementary to a target mRNA. On entering the cell, these fragments bind to cellular proteins to form an RNA-induced silencing complex. Within the complex, the two RNA strands separate, and the whole complex is guided to the target mRNA by the complementary sequence on the siRNA. On binding to the target, the complex cleaves and completely degrades the target mRNA, abolishing expression of the encoded protein.

This highly efficient 'knockdown' effect can be used to block expression of any specific protein rapidly, precisely and stably. Expression blocking is normally around 70%, which means

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that the system can be used on essential genes without lethal effects, and is controlled and reversible. As a result, RNAi has rapidly gained popularity as a technique for producing stable loss-of-function mutant model organisms, from nematodes to rodents, for use as research tools and disease models in drug target discovery and validation.

This has precipitated the first RNAi revolution – researchers can now choose any gene of interest (including those encoding proteins of unknown function), construct a siRNA and observe the effects when expression is silenced in living cells or a knockdown animal model. An RNAi knockdown model can be produced in a fraction of the time and cost required for previous antisense 'knockout mouse' technologies, and as a result these have been largely superseded by RNAi-based systems in a remarkably short period of time. As a result of its flexibility and reversibility, this type of system lends itself readily to HTS to identify not only functions of unknown genes but also possible targets for future drugs. If silencing a particular gene has a therapeutic effect, it is probable that a drug acting on the gene product will have a head start on its way to the clinic.

A new class of drug

Naturally, it has not escaped the notice of drug developers that the siRNAs producing therapeutic effects in models are prime drug candidates in themselves, and several specialist companies have sprung up to exploit this (Figure 1). Theoretically, a siRNA drug can be designed and developed against virtually any protein target, whether druggable by conventional molecules or not, and its precise mechanism of action reduces the potential for side-effects. The only major obstacles are delivery and intracellular stability of the siRNA agents – these issues are being tackled using vectors, protein or liposome conjugates and RNA modification. The broad spectrum of possibilities for RNAi therapeutics is illustrated by the wide variety of lead indications currently under investigation (Figure 2).

From Figure 1, the current major player in RNAi therapeutics is evident – Alnylam, founded in 2002 by siRNA pioneer Thomas Tuschl and Nobel laureate Philip Sharp. This impressive set of credentials was further strengthened by the acquisition of valuable RNAi patent rights through a merger with Ribopharma, making Alnylam a formidable player in this sector. The ambitions of the company are reflected in their offbeat choice of name; Alnylam is the central star on the belt of the constellation Orion (the hunter) with a luminosity that is 250,000 times greater than the sun.

Alnylam's lead project is an anti-vascular endothelial growth factor (VEGF) siRNA for age-related macular degeneration (AMD), which is expected to enter the clinic in 2005 in collaboration with Merck. The company also has a rich variety of back-up projects targeting a wide selection of indications, some notably involving targets previously deemed undruggable by conventional small molecules. A prime example of this is a siRNA targeting

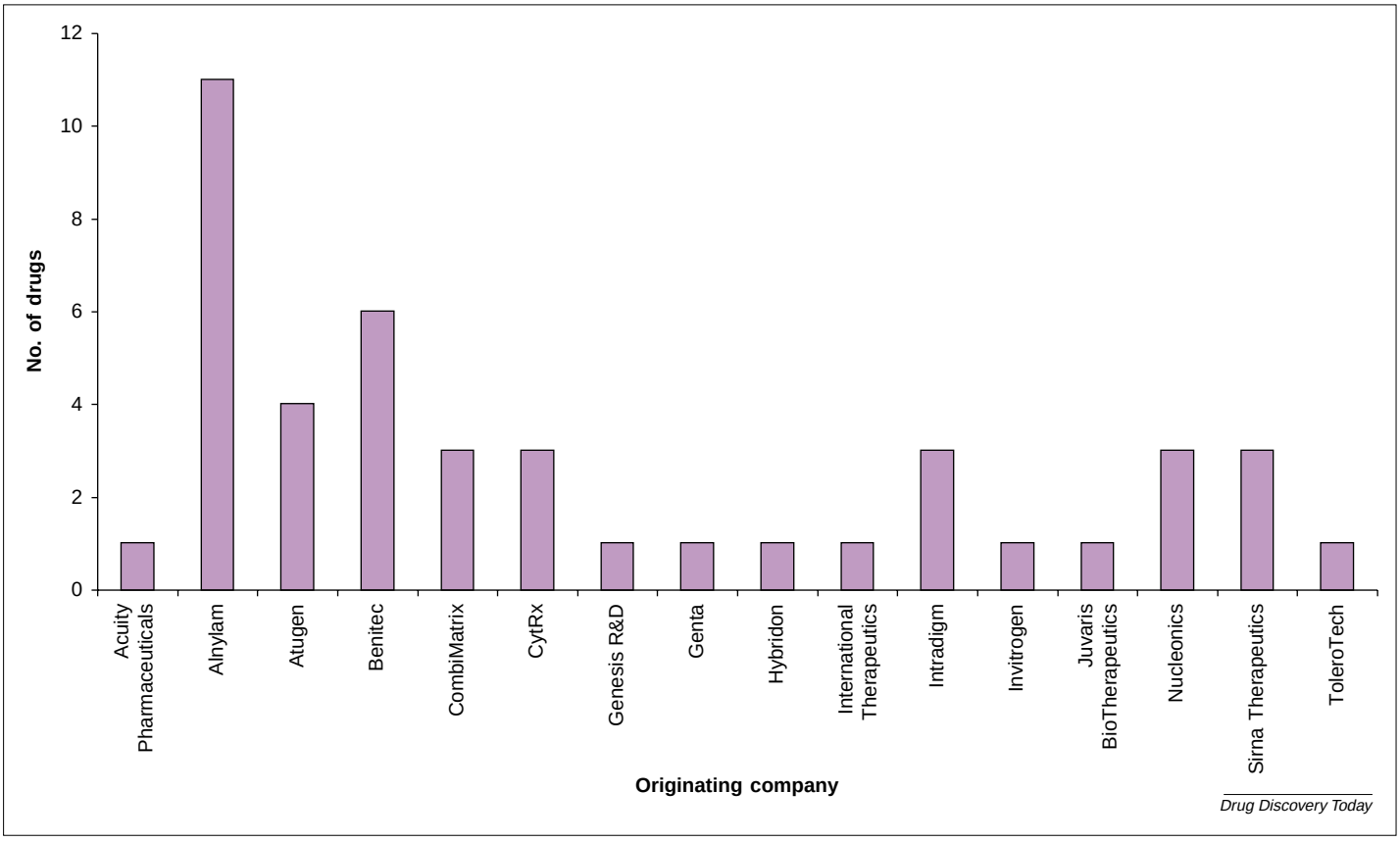

FIGURE 1

Companies developing RNAi therapeutics. Data courtesy of Pharmaprojects.

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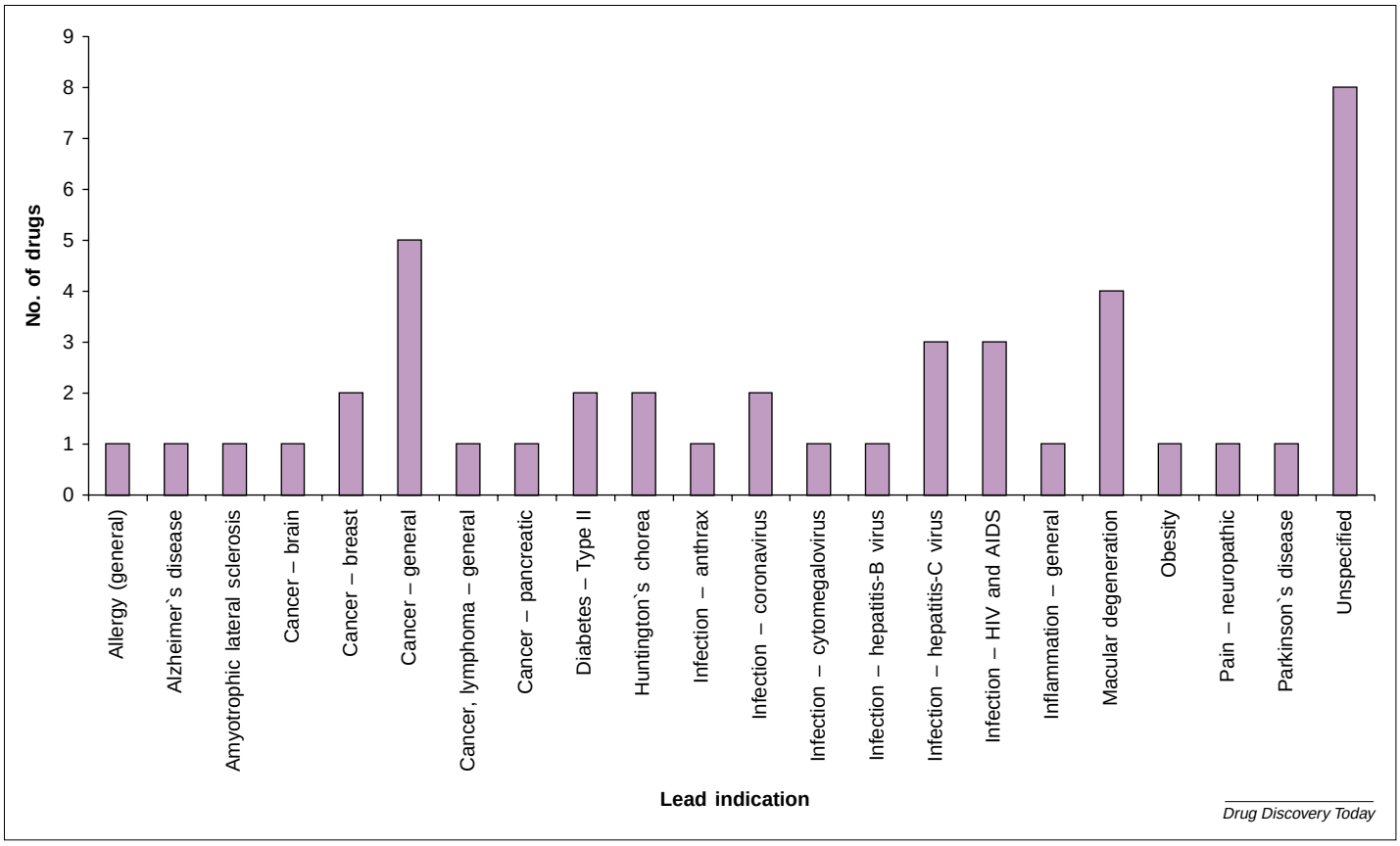

FIGURE 2

Indications targeted by current RNAi therapeutic projects. Data courtesy of Pharmaprojects.

α-synuclein, an important disease-modifying target for Parkinson's disease.

The more prosaically-named Sirna Therapeutics has a somewhat different history. It was created when Ribozyme Pharmaceuticals shifted its pipeline away from ribozyme therapies and jumped on the RNAi bandwagon, necessitating a name change. However, the time spent on ribozymes was not wasted – the considerable expertise of the company in RNA modification for therapeutic use should stand it in good stead for the development of stable siRNAs suitable for the clinic. Sirna's lead compound, Sirna-027, also an anti-VEGF siRNA, is currently entering Phase I for AMD.

The Australian company Benitec has carved an important niche for itself in RNAi using DNA vectors rather than oligonucleotides, and holds a dominant patent position in this area. The technology developed by the company is a version of gene therapy, which could have advantages over siRNA oligonucleotides in delivery, stability and

TABLE 1

RNAi therapeutics in late preclinical development

Product	Company	Indication	Status
Sirna-027	Sirna Therapeutics	Macular degeneration	C1 underway
Cand5	Acuity Pharmaceuticals	Macular degeneration and diabetic retinopathy	C1 underway
ALS RNAi therapy	CytRx	ALS	C1 due 2005
BLT-HCV[a]	Benitec	Hepatitis-C	C1 due 2005
Ocular siRNA therapy	Alnylam and Merck	Macular degeneration	C1 due 2005
RNAi HIV therapy[a]	Benitec and City of Hope	HIV and AIDS	C1 due 2005
RNAi	Genesis R&D	Allergy	C1 due 2006
BLT-HIV[a]	Benitec	HIV-related lymphoma	IND filing due 2005
ToleroVax[a]	ToleroTech	Autoimmune disease and transplant rejection	IND filing due 2005
Diabetes therapy	Atugen	Type II diabetes	IND filing due 2005–2006

Data courtesy of Pharmaprojects. [a]Denotes gene therapies using vectors – other products are synthetic oligonucleotides. Abbreviations: ALS, amyotrophic lateral sclerosis; C1, Phase I clinical trials; HCV, hepatitis-C virus; IND, Investigational New Drug.

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efficacy; however, because the gene therapy sector itself has been fraught with difficulties, commercialization is no more certain than with other RNAi strategies.

There are several key RNAi projects in or close to the clinical stage (Table 1). It is interesting to note that the race to be the first to enter clinical trials has been hotly contested between several VEGF-targeting siRNAs against the same ocular disorder – AMD, for which there is no current therapy. Also noteworthy, the first RNAi project to enter the clinic has not come from one of the major players but from a small Philadelphia-based company – Acuity Pharmaceuticals. Acuity's anti-VEGF oligonucleotide, Cand5, entered Phase I safety trials in AMD patients in October 2004 and is expected to complete this phase of clinical trials in 2005.

However, the bigger players have been hot on its heels. Sirna Therapeutics has now followed suit, commencing its first Phase I trial for Sirna-027 in November 2004. Not to be outdone, its major rival Alnylam has its own anti-AMD siRNA ready to begin trials in 2005. It will be interesting to see which project will be the winner of the next race – the first to make it to market.

A gold rush?

Many small companies are already clamouring to join the RNAi bandwagon. If the technology is not scuppered by safety issues or delivery problems, a huge expansion in the RNAi therapeutics sector is likely to occur over the next 2–3 years. However, the gene therapy debacle must be remembered. Gene therapy was hailed as a panacea and its popularity burgeoned during the past decade, only for the sector to dissolve into disappointment and frustration as efficacy and safety concerns halted project after project, with only one minor marketed product emerging thus far.

The human genome project has given RNAi developers a gold mine of untapped therapeutic targets on which to test their new technology. Unhampered by the need to develop small molecules through traditional means, and with RNAi technology itself generating screening models for each new target, the generation of actual drug candidates seems almost too easy. This might be a rare example of a newcomer living up to its advance publicity. However, the moment of truth will be the arrival of the first RNAi drug on the market, and its commercial success. Only then will RNAi overcome the most important target for silencing – the sceptics.

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YBF 2004: welcome to the future

Darren R. Flower, Edward Jenner Institute for Vaccine Research and **Jon Rees,** UK Bioinformatics Forum

Second annual Young Bioinformaticians Forum (YBF)
Said Business School, Oxford, UK
20th October 2004

In the past, provision of training in bioinformatics has been nebulous and haphazard, with courses varying dramatically in content and context: some emphasize the 'bio' aspect of the subject, whereas others focus on the informatics side. Bioinformatics is more than writing a Perl script, and much more than simply running BLAST (basic local alignment search tool). Fortunately, bioinformatics training has now changed beyond all recognition, becoming altogether more rigorous and has even begun to infiltrate the undergraduate curriculum.

As part of that process, the second annual *Young Bioinformaticians Forum* (YBF) one-day meeting was held on 20 October 2004 at the Said Business School (Oxford, UK). Run as a joint venture between the UK Bioinformatics Forum, which recently launched a new web portal for bioinformatics news, discussion and events (www.bioinformaticsforumuk.net), and the Royal Society of Chemistry Molecular Modelling Group, YBF 2004 used the modest success of last year's inaugural meeting as a foundation to build a more substantial and impressive event. YBF 2004 also drew support from the Said Business School, the South East England Development Agency and the Intermediary Technology Institute (Life Sciences). The meeting was also supported by its main media partner – BioMedCentral.

Theme and variation

The meeting was divided into three main themed sessions: (i) microarrays and clustering; (ii) systems biology and beyond; and (iii) protein evolution. Additional presentations by Martin Blythe (Edward Jenner Institute for Vaccine Research), who described research on the prediction of antibody epitopes, and Xueping Quen (University of Edinburgh, UK), who illustrated research on predicting and

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