

RNAi – A Glass Half-Full

Emile Bellott, a consultant in drug development, explores the buzz surrounding RNAi



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Gene silencing by RNA interference (RNAi) has captured the imagination of Wall Street and a new generation of biotech entrepreneurs. Rarely has there been so great a buzz surrounding a new therapeutic paradigm, including multi-billion dollar deals, venture capital funding of numerous start-ups and a Nobel Prize – all before the first marketing approval.

Enthusiasm in the industry for RNAi is encouraged by the notion that this may be the long-sought ‘holy grail’ of the genomics revolution. RNA interference is a method that can, at once, validate a target gene and lead directly from a gene to a drug. Scientists are intrigued by the idea that the discovery process could be encapsulated in a computer algorithm, whose input is genomic sequences. In practice, however, the discovery and development path is more complex. It will remain a human insight-intensive process, whose productivity is amplified by computation. In fact, the apparent simplicity of the RNAi phenomenon belies the considerable development of ancillary technologies required to advance and market a safe and efficacious therapeutic drug.

The advantages of the RNAi approach are groundbreaking: short timeline to target identification and validation; rapid realisation of *in vivo* proof-of-principle; the likelihood that animal models are predictive; the promise of specificity; harnessing an indigenous mechanism; silencing genes of interest, and so on. On the other hand, the obstacles to developing and marketing a therapeutic drug are manifest and challenging: formulation and delivery; uptake; intracellular localisation; PK/PD; tissue and cell-type specificity; off-target effects; potential for immunogenicity; CMC challenges; and regulatory hurdles, among others.

The industry has recognised this situation as a ‘glass half-full’. The programmes closest to the clinic have carefully adopted strategies that tend to avoid or minimise the most difficult obstacles on the path to market.

This article presents an overview of RNAi technology in a business strategy context, and discusses the issues involved in developing a commercially viable product. Several examples of current therapeutic programmes are discussed in the framework of Graham and White’s *Harvard Business Review* article, in which they delineate business and scientific criteria for commercially successful technological innovations.

Radical scientific innovations are a profound challenge to the pharmaceutical and biotechnology industry – on the one hand opening up new opportunities to serve the market; on the other,

forcing a rethinking of existing business paradigms and operating assumptions. RNAi is such an innovation. Already, it has proven its value as a research tool in target validation for conventional small-molecule drugs and biologics. Ironically, RNA interference has become the new paradigm for innovative drug discovery, while simultaneously becoming an essential component of the discovery and target validation process in the development of small-molecule drugs, by classical screening and rational design methodology.

In the short time since the initial demonstration of RNAi in animals, numerous companies have been launched in order to exploit the direct therapeutic potential. Billion-dollar deals have been made, and a Nobel Prize has been awarded.

The intense commercial interest in this technology highlights its strategic importance. In the past decade, much has been made of the need for additional clinical candidates to fill pharmaceutical pipelines: expiration of patents; increasing pressure for ROI; and the trend toward discovery and development partnerships with smaller innovator companies and academia. Add to this ‘perfect storm’, until recent months, relatively easy liquidity in public and private financial markets, which propels new start-up companies.

WHAT IS RNA INTERFERENCE?

When a double-stranded small interfering RNA (siRNA) is administered therapeutically, the normal translation of a specific messenger RNA to protein product is abrogated and the further expression of the relevant gene product is effectively blocked. This gene silencing serves as a versatile modality for therapeutic intervention. It also operates routinely in the normal lifecycle of cells, to regulate gene expression. Scientists speculate that this gene-regulatory mechanism first appeared in higher animals as a defense against viruses.

Therapeutic application of RNAi was enabled by the fundamental discovery of the RNA interference phenomenon – first in plants, then in worms and finally in higher animals. The mechanism of RNA interference has been described extensively in the recent literature. In this phenomenon, expression of a gene product (protein) is ‘silenced’ or ‘knocked-down’ by harnessing a natural cellular mechanism to destroy the targeted messenger RNA. RNAi is mediated by small RNA oligomers – approximately 21 base pairs appear to be optimal. Synthetic oligos are administered as a duplex, one of whose strands, the ‘guide’ (antisense), is complementary to a subsequence of the targeted

mRNA, otherwise, the rest of the natural process is the same. As the RNA interference event proceeds, the guide strand is held within the heterotrimeric RNA-induced silencing complex (RISC) by which it serves as a template for hybridisation of the complementary mRNA to present it for degradation by cleavage.

SMALL INTERFERING RNA AS A THERAPEUTIC DRUG

An RNA interference drug differs in important ways from small-molecules, and therapeutic antibodies. In the most optimistic case, this technology offers stunning advantages (see ‘Advantages and Challenges of RNAi’). These include:

- ◆ All gene products can be targets, in principle – any gene whose sequence is known can be silenced, including many targets that are not ‘druggable’ by small molecules or protein therapeutics
- ◆ The discovery and early development timeline is faster – hundreds of analogues can be tested and proof-of-principle demonstrated *in vitro* and *in vivo* within a few months of programme initiation
- ◆ Detailed knowledge of the target gene product is not necessary – the minimum information needed is the gene sequence, and a rationale for the target’s role
- ◆ Excellent specificity – in theory, it is possible to find a unique 21-base nucleotide within the gene of interest
- ◆ Long duration of action – multi-day sustained duration of gene silencing by siRNA has been observed
- ◆ Ease of analogue synthesis – synthesis of nucleotides is modular and amenable to automation. Bench scale synthesis of oligos at the discovery stage is routine, and small-scale suppliers abound

RNAi AS A RADICAL TECHNOLOGICAL INNOVATION

In their *Harvard Business Review* paper, ‘How to Spot a Technological Winner’, Graham and White discuss the important factors that influence the successful outcome of a radical new innovation in the marketplace. The present intensity of the commercial race in RNAi reflects a belief within the biotech community that the interplay and trade-off of business drivers and technical hurdles is favourable. Graham and White have grouped these success factors into two large categories – ‘Technology Potency’ and ‘Business Advantage’ (see Figure 1, adapted from their article).

Graham and White’s formulation concludes that enterprises that are able to harness the basic technology to successfully meet the needs of the marketplace will succeed in their drive to commercial success. They will do this by successfully recognising and exploiting both the enabling and diluting attributes of the innovation. In the case of RNAi, the difficulties and opportunities inherent in exploiting this technology are summarised and grouped according to the stage of the classic drug development cycle (Table 1, see page 46).

INVENTIVE MERIT

Memorable and successful innovations employ scientific principles in a new way, superseding limitations of the prior art. Usually, at the same time, the new method has its own constraints that must be

overcome, or avoided. In the most ‘inventive’ new discoveries, the possibilities enabled outweigh the new constraints imposed.

The core hypothesis of RNA interference therapeutics is to silence gene expression at the messenger RNA level. This is in contrast to conventional small molecule and biological therapeutics, where the activity of target proteins (already expressed) is inhibited by interaction with the drug molecule. By way of example, a small molecule could inhibit an enzyme. RNAi would block the expression of the enzyme in the first place. In either instance, the resulting absence of enzymatic activity could mediate a biological effect.

Therapeutic approaches have paralleled the growth of our understanding of the details of cellular mechanisms and the development of other enabling technologies. The ability to make potent and specific intervention in gene expression is predicated on new understanding of the RNA interference phenomenon, as well as the rapid development of gene sequence information on a large scale for man and other important model organisms. Other enabling developments in informatics, manufacturing automation, analytical techniques and chemical synthesis were all necessary preconditions to the current state-of-the-art in RNAi technology.

When these technological factors are brought into play, it is possible to design, test and validate the silencing of a designated gene target of interest, and to rapidly enumerate and synthesise hundreds of candidate analogues within a few months of programme go-ahead. The science is not separate from market realities – the initial choice of target will depend on the capabilities of the RNA interference phenomenon and the ancillary formulation and delivery technology, to meet a marketplace-acceptable product profile and gain regulatory approval.

With the new wealth of genomic targets available for exploitation by RNAi, early lead development places new demands on the R&D organisation. First, new skill sets. The basic design paradigm is informatics-driven. Within a target gene, all possible 21-base sequences are enumerated and exhaustively compared within the relevant genome database for matches (FASTA or other methods). The high theoretical specificity of RNAi is due to the high probability of finding at least one or more unique 21-base subsequence(s) within the gene and its 5’UTR.

Following this simple vetting procedure, additional proprietary informatic filters are applied to prioritise candidates according to predicted binding specificity within the RISC complex: potency; hybridisation efficiency; privileged substructures; immunogenic potential; and other predicted biological properties. These

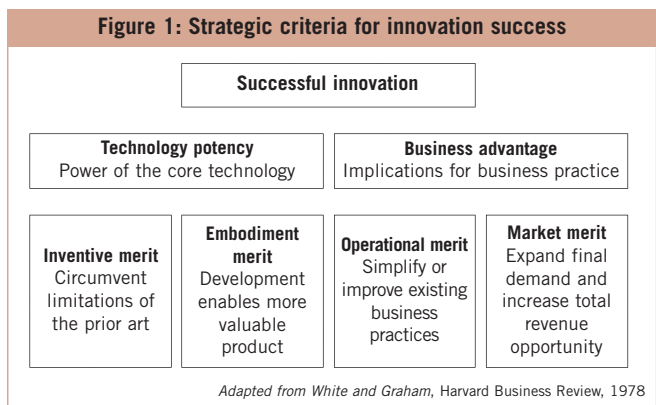


Table 1: Evaluation of the factors contributing to innovation success

The columns are the four factors of Graham and White (Inventive Merit, Embodiment Merit, Operational Merit, and Market Merit). These four factors, taken together, comprise the main drivers of Technology Potency and Business Advantage.

Factors Relating to Innovation Success				
Stage of drug development cycle	Inventive (constraints added/lifted)	Embodiment (enhancement/dilution)	Operational (business simplified /complicated)	Market (demand expanded/ constrained)
Discovery	<ul style="list-style-type: none"> ◆ All possible targets ◆ Simple <i>in vitro</i> discovery ◆ New skill set ◆ Automation 	<ul style="list-style-type: none"> ◆ Simple chemistry at bench scale ◆ Simple screening methods 	<ul style="list-style-type: none"> ◆ Fast development cycle 	<ul style="list-style-type: none"> ◆ High potency ◆ High selectivity ◆ Specificity
Validation and early lead development	<ul style="list-style-type: none"> ◆ Rapid validation ◆ Off-target effects ◆ Immunogenicity ◆ Bioinformatics ◆ Bioanalytical 	<ul style="list-style-type: none"> ◆ Concurrent development of delivery system ◆ Chemical modifications 	<ul style="list-style-type: none"> ◆ Easy synthesis of analogs for research 	
Delivery system		<ul style="list-style-type: none"> ◆ Uptake ◆ <i>In vivo</i> stability ◆ Systemic delivery ◆ Tissue specificity ◆ Cell type specificity ◆ Intracellular localisation ◆ Renal clearance 		
Synthesis and scale-up		<ul style="list-style-type: none"> ◆ Large scale purification ◆ Automated synthesis ◆ QC and analytical development 	<ul style="list-style-type: none"> ◆ Solid-phase synthesis ◆ When to invoke GMP 	
Non-clinical development		<ul style="list-style-type: none"> ◆ <i>In vivo</i> stability ◆ PK/PD ◆ Shelf stability ◆ Formulation 		
IND			<ul style="list-style-type: none"> ◆ Regulatory concerns 	
Clinical development			<ul style="list-style-type: none"> ◆ Immunogenicity ◆ Off-target effects 	<ul style="list-style-type: none"> ◆ Formulation and delivery
Manufacture, marketing and distribution			<ul style="list-style-type: none"> ◆ Shelf-life of drug product ◆ Industrial-scale cGMP synthesis 	<ul style="list-style-type: none"> ◆ High potency ◆ High selectivity ◆ New indications

proprietary filters are often empirical rule-based algorithms. Finally, each of the candidate oligonucleotides must be made and screened *in vitro* and *in vivo*.

This filtering and other downstream adjustment and re-testing is a consequence of unanticipated attributes of RNAi that need to be overcome in discovery or development. Off-target effects due to RNAi, triggered by the sense strand of the siRNA duplex, include: weak off-target binding of the RISC complex with other mRNAs via as few as seven base pairs; and immunostimulatory response, for example induction of interferon alpha through toll-like receptors.

The chemical synthesis of RNA candidates is rapid and straightforward. However, the special property-modulating modifications to make them endonuclease-resistant, tighter-binding and less immunogenic require a different breed of nucleoside-chemistry skilled medicinal chemists and structural biologists to support the work. They also define and prepare a stockpile of proprietary modified-nucleoside building blocks. Still, the rapid turnaround of oligonucleotide synthesis is in contrast to the laborious analoguing of small molecules or the long time required for cell line development and scale-up in biotherapeutics, like monoclonal antibodies.

Development-enabled nucleotide candidates can be made by overcoming the constraints inherent in the molecular biology of

RNAi: immunogenic potential; low stability; and off-target effects. These are mainly resolved at this stage, or in early development, by adjustment of the nucleotide sequence and incorporation of non-natural nucleosides in the chain.

One consequence of the paradigm shift in discovery and early development is the need to develop a new team through extensive hiring of new skills or outright acquisition of smaller companies, and by extensive in-licensing of intellectual property.

EMBODIMENT MERIT

An invention, no matter how creative, generally requires additional engineering to reach its ultimate marketable form. The development required may offer opportunities to create a more valuable product embodiment than originally conceived. At the same time, this sometimes comes at the considerable cost of developing essential ancillary technologies to achieve the best potential outcome. For management, the primary concern is to see that the value is maximised overall.

The putative drug candidate becomes a valuable commercial product once it has demonstrated safety and efficacy and has been approved for human use. Advancing from discovery to lead development to clinical development means ultimately that a drug product must be made that safely fulfils the commercial requirements of the therapeutic product profile.

A great deal of excitement in the early days of RNAi was based on the unbounded prospects of low side effects, and unprecedented efficacy. The literature reveals that with added work in the discovery and development phase, these goals have, in many cases, been realised.

Efficacy, particularly with *in vivo* models, is dependent on drug uptake, delivery, renal and metabolic clearance, tissue and cell-type specificity, and intracellular localisation. Optimisation of uptake and bioavailability of nucleosides is difficult. A typical siRNA drug molecule is over 12,000 in molecular weight, carrying 40 or more negative charges, hydrated and highly polar. Successful absorption of such a molecule or penetration of biological membranes, like cell walls, would be tenuous at best. In plasma, oligos are rapidly degraded and eliminated. Addressing these problems has become the major enabling activity of any RNA therapeutic drug programme.

Maintaining stability of a small RNA duplex – indeed any nucleoside – from dosing to intracellular locus of action is a vexing problem. It is understood that naked RNA is rapidly degraded ($t_{1/2}$ less than a minute) by endonucleases in blood plasma. This may be ameliorated somewhat by 2' modification of the nucleosides at the design stage and by the choice of protective delivery formulation as a component of the development cycle. Once endonuclease resistance has been improved, renal filtration can be reduced by conjugation of the oligo with polyethylene glycol (PEG).

Furthermore, since RNAi results in substantially complete silencing of gene expression, it is preferable to achieve tissue and cell-type specificity. It is undesirable to shut down target gene expression in cells where it is not therapeutically relevant or may lead to undesired side effects. It is known that gene silencing by RNAi is not an equilibrium effect. Thus, when siRNA guide strand is incorporated into the RISC complex, the therapeutic effect endures for the life of the turnover of RISC – namely a timespan of a matter of weeks.

These myriad formidable problems in non-clinical development are being addressed by formulation and delivery systems. Such expedients as conjugates and peptide labelling are current areas of delivery research. Formulation – particularly liposomes and nanoparticles – is a large and essential element of the early development programme at all RNAi companies. All new RNAi drug programmes require the commitment of substantial resources to these areas.

Anticipating progression in clinical development, pharmacokinetic and pharmacodynamic considerations have been addressed by medicinal chemistry and concurrent engineering of the delivery system. For example, the use of a multi-component liposomal formulation called 'stable nucleic-acid lipid particles' (SNALP) has shown plasma half-life of greater than 12 hours and has demonstrated drug product shelf life of up to two years at 4°C. Other conjugation methods have successfully achieved tissue-specific delivery and target silencing *in vivo* of a matter of weeks.

Another area of importance to the development stage is economical manufacturing scale-up. Bench-scale synthesis and analysis is not a problem in the discovery and early development phases. Most of the late development and clinical supply of API is outsourced to a few contract manufacturers – Avicia, Dow and Agilent, for example – who have established the specialised

expertise to produce small oligonucleotides, including double-stranded RNA under cGMP conditions. The synthesis follows the classic phosphoramidite synthesis protocol on solid phase, with added proprietary know-how. It is a convergent synthesis paradigm, finished by annealing of the two strands at the last step. Purification is made more difficult by the fact that RNA duplex formation is not thermodynamically favoured.

Current attention is focused on process improvements that improve purity and yield and cut cycle time. As companies approach a higher scale, there is increased interest in development of efficient solution-phase synthesis. Until now, multi-kilogram scale-ups have been accomplished on resin. Other important issues include analytical method development; specifications and sourcing of raw materials; minimisation of large-scale chromatographic purification if possible; and choice of the step where GMP protocols commence.

In CMC, the FDA has not provided regulatory guidance on RNA therapeutics. Work at present is relying on recommendations and analytical methods developed earlier for antisense oligo programmes at ISIS Pharmaceuticals. The prevailing regulatory paradigm is to view oligos as 'small molecules' rather than biologicals.

Delivery system manufacture is generally partnered or in-licensed from specialist companies in the liposome or nanoparticle field, although the development work is closely coupled to the actual drug R&D, with substantial work being done in-house.

OPERATIONAL MERIT

An enterprise's existing business practices and operations may be simplified, complicated or superseded by the requirements of the new innovation. The magnitude of these changes to business operations will affect the cost structure of the enterprise (both cost of goods and capital employed in production and marketing).

Developing a therapeutic drug based on RNAi simplifies the discovery and early development cycle, but increases the complexity of business operations to develop the actual drug product and dosage form (API and delivery system) that is safe and efficacious.

Due to the relative newness of the technology, regulatory hurdles are greater, particularly for a first-in-class drug product. Recent adverse events in antibody therapeutics tend to raise the bar for RNA therapeutics, where immunogenicity is a known concern, and for which there is not yet large-scale clinical experience. Shelf stability will need to be conclusively demonstrated, in light of the understood lability of nucleotides. Although these are challenging issues, they represent business as usual for a pharmaceutical development company with clinical development experience.

The intellectual property landscape of RNAi is still being defined. Many patent applications are published but not yet allowed. However, some fundamental patents have been issued. The rights to the major RNAi intellectual property are concentrated in a few enterprises. Extensive cross-licensing will be needed; particularly for small innovator companies to secure freedom to operate to develop RNAi drug products. The prevailing business model to license tickets to any player that wants to get into RNAi therapeutics assures additional intellectual horsepower will be applied to the resolution of embodiment problems such as delivery,

immunogenicity and off-target effects. But newcomers will increasingly have to find a niche or special technology in order to compete profitably.

MARKET MERIT

Financial return to the owners of the business is a primary measure of a programme's success. Thus, in judging success, management looks at the extent to which an innovation offers an opportunity to expand final demand in the marketplace and to increase business revenues.

Financial market success of the public RNAi companies has provided validation of RNAi therapeutic drugs as a valuable business proposition. If the promises of the technology are fulfilled and the diluting factors are overcome, as they seem poised to do, it is likely that these products will succeed in the marketplace. Overall, total demand for pharmaceutical drugs will be expanded because RNAi opens up additional targets and indications that heretofore had been inaccessible. In any individual case, a product may fail to achieve optimal market share as a consequence of product profile or pricing factors. The most prevalent strategies for business success are to advance products that solve unmet or underserved medical needs, and that address indications where the known limitations of RNAi are least likely to derail development plans and can offer an acceptable product profile.

For example, some of the current early clinical programmes have taken this approach. In each case, one can speculate that the choice of target and indication avoids the drawbacks of delivery, stability, tissue or cell-type specificity and off-target effects:

- ◆ Age-related macular degeneration (AMD) – avoids systemic delivery problems
- ◆ Hypercholesterolemia – delivery on first pass to minimise stability problems, conjugates or engineered liposomes for targeting, single cell type locus
- ◆ Respiratory syncytial virus (RSV), hepatitis C-virus (HCV) and influenza – non-host targets, to avoid issues with cell or tissue specificity
- ◆ RSV – direct delivery to the site of action; obviates the problems of systemic exposure
- ◆ Acute renal failure (ARF) – simplified delivery

According to news reports, the first investigational new drug application (IND) for a systemically delivered RNAi therapeutic will be filed in late 2007, by Alnylam. This will be either ALN-PCS01, for hypercholesterolemia, or ALN-VSP01, for liver cancer. Significantly, these higher-risk programmes follow the conservative, pioneering path of the lower technical risk indications, above – wherein many regulatory and operational issues will have been addressed.

Note that these programmes appear to match a product profile, for which the designated delivery systems appear adequate. In these indications, inhalation, injection or infusion are acceptable delivery modes. On the other hand, an RNAi drug targeting a chronic disease, for example type II diabetes or elevated blood pressure, face market forces to meet the gold standard of oral dosing that prevails in the leading drugs. It may be, however, that the prospect of sustained duration of action would provide a more

Advantages and challenges of RNAi

Is the glass half-full or half-empty? Gene knockdowns are routine, at the bench. Recent literature shows a greater understanding of the factors that make an efficacious and safe RNAi therapeutic drug. But many daunting engineering hurdles remain to be solved before the first RNAi drug is approved for manufacture and marketing. The greatest hurdles are delivery and distribution, stability and specificity. The industry is taking a cautious view of safety issues, particularly since any of the current clinical candidates are potentially first-in-class and under the microscope of the investment community as well as regulators.

Advantages and pay-offs	Challenges to be overcome
Rapid target validation	Uptake and bioavailability
Non-druggable targets (in the classical sense) may be targeted	Distribution (systemic delivery)
High potency	Intracellular localisation
High specificity	Tissue specificity
Long duration of action	Pharmacokinetics
Potential for low toxicity	<i>In vivo</i> stability
	Off-target effects
	Immunogenic potential
	API manufacturing under cGMP
	Formulation
	Shelf stability
	Regulatory

advantageous product profile, in terms of patient compliance and clinical outcomes, thus justifying injection or infusion.

The field of diagnostic applications is a growing area of interest. Since this can occur *ex vivo*, much of the embodiment dilution – issues relating to delivery and side effects – is mitigated.

RNAi therapies can expand the market for pharmaceutical drugs by opening up new disease indications heretofore not druggable, and by providing improved side effect profiles and dosing regimens in existing indications. Early corporate players, who have secured rights to the fundamental intellectual property, will gain the added advantage of licensing and partnership revenues, to the competitive disadvantage of the later entrants.

CONCLUSION

Evaluation of the technology potency and business advantage of RNAi therapeutics in light of the recent activities of innovator pharmaceutical and biotechnology companies suggests that RNAi technology is on a trajectory for success. The technology is advantageous in discovery of NCEs and has successfully created several robust pipelines aimed at commercialisation. The difficulties in delivery, immunogenicity and off-target effects have all been addressed with tentative solutions and vigorous proprietary research programmes. At the same time, candidates in early clinical phases have taken the conservative approach to defining the indication, target and product profile for these early products.

Adopting a business model that allows for multiple non-exclusive licenses of the fundamental patents is actually beneficial to the marketplace, the industry and the licensor. It allows for many more investigators to arrive at new solutions to the most difficult enabling problems of therapeutic RNAi, and leads to the development of an expanded talent pool.

Significantly, the larger, more traditional pharmaceutical companies, whose success has heretofore been based on small-molecule therapeutics, are less able to compete in the RNAi

marketplace than biotechnology companies. This deficit in skill-sets, biologics experience and IP access has driven the large deals in RNAi, antisense, aptamers, and miRNA. Alnylam – Novartis and Roche; Silence – AstraZeneca; ISIS – BMS, Ortho-McNeil/J&J; Archemix – Elan, Merck, Serono, Pfizer, Takeda; and Sirna – Merck.

Finally, the collective activity in larger pharmaceutical companies and many start-ups has provided a growth opportunity for outsourced manufacturing, suppliers, drug delivery technology companies and CROs. ♦

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References

1. Aigner A, Delivery Systems for the Direct Application of siRNAs to Induce RNA Interference (RNAi) *In Vivo*, *Journal of Biomedicine and Biotechnology* 1, 2006
2. Aigner A, Gene Silencing Through RNA Interference (RNAi) *in vivo*: Strategies Based on the Direct Application of siRNAs, *Journal of Biotechnology* 124, p12, 2006
3. Brownlee C, Discovering The Building Blocks of RNA Interference, *ACS Chemical Biology* 1, p126, 2006
4. Bumcrot D, Manoharan M, Koteliansky V and Sah DWY, RNAi Therapeutics: A Potential New Class of Pharmaceutical Drugs, *Nature Chemical Biology*, 2, pp711-719, 2006
5. DeFougerolles A, Manoharan M, Meyers R and Vornlocher H-P, RNA Interference *In Vivo*: Toward Synthetic Small Inhibitory RNA-Based Therapeutics, *Methods in Enzymology* 392, pp278-296, 2005
6. DeFougerolles A, Vornlocher H-P, Maraganore J and Lieberman J, Interfering with Disease: A Progress Report on siRNA-Based Therapeutics, *Nature Reviews-Drug Discovery* 6, pp443-453, 2007
7. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC, Potent and Specific Genetic Interference by Double-Stranded RNA in *Caenorhabditis Elegans*, *Nature* 391, p806, 1998
8. Glaser V, Improving Yields In Oligo Manufacturing, *Genetic Engineering and Biotechnology News* 27, May 15th 2007
9. Hamilton DP and Zimmerman R, Nobel Discovery Already Sparked Hunt For Drugs, *Wall Street Journal*, October 3rd 2006
10. Healy JM, Lewis SD, Kurz M, Boomer RM, Thompson KM, Wilson C and McCauley TG, Pharmacokinetics and Biodistribution of Novel Aptamer Compositions, *Pharmaceutical Research* 21, p2,234, 2004
11. Hornung V, Guenther-Biller M, Bourquin C, Ablasser A, Schlee M, Uematsu S, Noronha A, Manoharan M, Akira S, de Fougerolles A, Endres S and Hartmann G, Sequence-Specific Potent Induction of IFN- α by Short Interfering RNA in Plasmacytoid Dendritic Cells Through TLR7, *Nature Medicine*, 11, p250, 2005
12. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M and Stoffel M, Silencing of MicroRNAs *in vivo* With 'Antagomirs', *Nature* 438, p685, 2005
13. Kumar P, Wu H, McBride JL, Jung K-E, Kim MH, Davidson BL, Lee SK, Shankar P and Manjunath N, Transvascular Delivery of Small Interfering RNA to the Central Nervous System, *Nature* 448, p39, 2007
14. Li CX, Parker A, Menocal E, Xiang S, Borodyansky L and Fruehauf JH, Delivery of RNA Interference, *Cell Cycle* 5, pp2,103-2,109, 2006
15. Manoharan M, RNA Interference and Chemically Modified siRNAs, *Nucleic Acids Research Suppl* 3, pp115-116, 2003
16. Mattes J, Yang M and Foster PS, Regulation of MicroRNA by Antagomirs, *American Journal of Respiratory Cell Molecular Biology* 36, p8, 2007
17. Pei Y and Tuschl T, On The Art of Identifying Effective and Specific siRNA's, *Nature Methods* 3, p670, 2006
18. Montgomery MK, Xu S, Fire A, RNA as a Target of Double-Stranded RNA-Mediated Genetic Interference in *Caenorhabditis Elegans*, *Proceedings of The National Academy of Sciences*, USA 95, p15,502, 1998
19. Robbins MA and Rossi JJ, Sensing the Danger In RNA, *Nature Medicine* 11, p250, 2005
20. Rossi JJ, Receptor Targeted siRNAs, *Nature Biotechnology* 23, p682, 2005
21. Schlee M, Hornung V and Hartmann G, siRNA and isRNA: Two Edges of One Sword, *Molecular Therapy* 14, p463, 2006
22. Shankar P, Manjunath N and Lieberman J, The Prospect of Silencing Disease Using RNA Interference, *Journal of the American Medical Association* 293, pp1,367-1,373, 2005
23. Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, Elbashir S, Geick A, Hadwiger P, Harborth J, John M, Kesavan V, Lavine G, Pandey RK, Racie T, Rajeev KG, Rohl I, Toudjarska I, Wang G, Wuschko S, Bumcrot D, Koteliansky V, Limmer S, Manoharan M and Vornlocher HP, Therapeutic Silencing of an Endogenous Gene by Systemic Administration of Modified siRNAs, *Nature* 432, p173, 2004
24. Tomari Y and Zamore PD, Perspective: Machines for RNAi, *Genes and Development* 19, pp517-529, 2005
25. Marques JT, Devosse T, Wang D, Zamanian-Daryoush M, Serbinowski P, Hartmann R, Fujita T, Behlke MA and Williams BRG, A Structural Basis for Discriminating Between Self and Non-Self Double-Stranded RNAs in Mammalian Cells, *Nature Biotechnology* 24, p521, 2006
26. White GR and Graham MBW, How to Spot a Technological Winner, *Harvard Business Review*, pp146-152, 1978
27. Wilson C and Keefe AD, Building Oligonucleotide Therapeutics Using Non-Natural Chemistries, *Current Opinion in Chemical Biology* 10, p607, 2006
28. Wolfrum C, Shi S, Jayaprakash KN, Jayaraman M, Wang G, Pandey RK, Rajeev KG, Nakayama T, Charrise K, Ndungo EM, Zimmermann T, Koteliansky V, Manoharan M and Stoffel M, Mechanisms and optimization of *in vivo* delivery of lipophilic siRNAs, *Nature Biotechnology* 25, p1149 2007
29. Xia J, Noronha A, Toudjarska I, Li F, Akinc A, Braich R, Frank-Kamenetsky M, Rajeev KG, Egli M and Manoharan M, Gene Silencing Activity of siRNAs With a Ribo-difluorotoluy Nucleotide, *ACS Chemical Biology* 1, p176, 2006
30. Xinag S, Fruehauf J and Li CJ, Short Hairpin RNA-Expressing Bacteria Elicit RNA Interference in Mammals, *Nature Biotechnology* 24, p697, 2006