



DRUG DISCOVERY

Outlook for RNAi, 2007

siRNA and miRNA in biology, diagnostics and therapeutics

By Dr CL Barton

 [Table of Contents](#)

Dr Cheryl L Barton

Dr CL Barton has over 10 years' experience as a practical R&D analyst with a leading pharmaceutical company and as a equity research analyst with a European Bank.

Dr CL Barton Ltd. aims to provide independent, tailor-made, pharmaceutical thematic research to investment houses. Where applicable the research reports combine independent scientific analysis with patients- and prescription-based models to forecast the potential sales growth of key developmental drugs and isolate the key drivers within the pharmaceutical sector. For more details visit www.pharmavision.co.uk

Copyright © 2007 Business Insights Ltd

This Management Report is published by Business Insights Ltd. All rights reserved. Reproduction or redistribution of this Management Report in any form for any purpose is expressly prohibited without the prior consent of Business Insights Ltd.

The views expressed in this Management Report are those of the publisher, not of Business Insights. Business Insights Ltd accepts no liability for the accuracy or completeness of the information, advice or comment contained in this Management Report nor for any actions taken in reliance thereon.

While information, advice or comment is believed to be correct at the time of publication, no responsibility can be accepted by Business Insights Ltd for its completeness or accuracy.

Table of Contents

Outlook for RNAi

Executive Summary 12

Introduction	12
Design and Production of RNAi effectors	13
RNAi Research and Applications	14
Delivering RNAi therapeutics	15
RNAi Therapeutics: Progress and the Future	16
miRNA: diagnostics and therapeutics	17
Regulatory issues and patents	18
RNAi markets and trends	19

Chapter 1 Introduction 22

Summary	22
Introduction	23
DNA to RNA to protein – a primer	23
Antisense technologies	24
RNA interference	28
Overview and significance	28
RNAi's mechanism	31
Post-transcriptional gene silencing (PGTS) by siRNA	31
miRNA pathways	32
Transcriptional gene silencing (TGS) by siRNA	34
Other non-coding RNAs	34
Advantages and disadvantages of RNAi	36
Report Outline	37

Chapter 2 Design and Production of RNAi effectors 40

Summary	40
Introduction	41
Design of RNAi effectors	41

siRNA design algorithms	42
Building silencing efficiency	45
Avoiding off-target effects	48
Chemical modification	49
Avoiding immunostimulation	50
Finding the right target sites	52
miRNA mimics	52
Production of RNAi effectors	53
Chemical Synthesis	55
siRNA pools	58
Dicer-substrate siRNAs (DsiRNAs)	60
Expression vectors	60
Plasmid expression vectors	62
Expression from a PCR product	64
Viral expression vectors	65
Conclusions	67

Chapter 3 RNAi Research and Applications 70

Summary	70
Introduction	71
Elucidating gene function	71
High throughput loss-of-function screening with RNAi	72
Choice of species and cell-line	73
RNAi reagents	74
Delivery method	80
Screening paradigm and format	81
Read-out methods	82
Building pathways	83
Investigating gene function <i>in vivo</i>	84
Applications of RNAi in drug discovery and development	88
Target discovery and validation	88
Optimization of drug therapy and development of personalized medicine	90
miRNA Research	91
miRNA Databases and Algorithms	92
Identifying miRNAs, their targets and function	93
Isolation and enrichment of miRNA	94
Detection and quantification of miRNA	94
Functional analysis	98
Conclusions	100

SARS		155
	Key RNAi players	156
Herpes Simplex Virus (HSV)		156
Other viral diseases		157
Respiratory diseases		157
Asthma and Chronic Obstructive Pulmonary Disease (COPD)		157
	Key RNAi Players	158
Cystic fibrosis (CF)		158
	Key RNAi players	159
Neurological diseases		159
Oncology		163
Angiogenesis		164
	Key RNAi players	164
Oncogenes		166
	Key RNAi players	166
Cardiovascular disease		168
	Key RNAi players	169
Metabolic disorders		170
Diabetes and Obesity		170
	Key RNAi players	170
Dermatology		172
	Hair Removal	172
	Key RNAi players	172
	Pachyonychia congenita and related disorders	173
	Key RNAi Players	173
Acute renal failure		174
	Key RNAi players	174
Acute hearing loss/ pressure sores ototoxicity		175
	Key RNAi players	175
Inflammatory Diseases		176
	Key RNAi players	176
Conclusions		177

Chapter 6 miRNA: diagnostics and therapeutics 180

Summary		180
Introduction		181
miRNA as a diagnostic tool		182
Companies developing miRNA-based diagnostics		183
	Rosetta Genomics	183
	Cepheid	186
	Stratagene	187

miRNA-based therapeutics	187
Companies developing miRNA-based therapeutics	189
Alnylam Pharmaceuticals	189
Asuragen Inc	189
Santaris	191
Sirna	191
Conclusions	192
Chapter 7	Regulatory issues and patents
	194
Summary	194
Introduction	195
Patents in RNAi	196
Seminal patents in RNAi	196
Alnylam	199
Sirna	201
Chemical modifications	201
DNA directed RNAi	202
Patents for specific RNAi targets	204
The future – more patent litigation?	205
MicroRNA	205
Tuschl III patents	206
Zamore and other patents	206
Regulatory considerations for RNAi therapies	207
Conclusions	207
Chapter 8	RNAi markets and trends
	210
Summary	210
Introduction	211
The RNAi market	213
Market size and future trends	218
RNAi suppliers: synthesis and reagents	219
Alliances with big pharma for RNAi in R&D	222
Delivery of RNAi therapeutics	224
RNAi-based therapeutics	225
Big pharma alliances for RNAi-based therapeutics	229
miRNA-based diagnostics	230
Conclusion	231

List of Figures

Figure 1.1:	Summary of antisense technologies other than RNAi	25
Figure 1.2:	Timeline of RNAi discoveries	29
Figure 1.3:	Significance of RNAi	30
Figure 1.4:	Mechanism of post-transcriptional gene silencing by siRNA	32
Figure 1.5:	Mechanism of miRNA mediated translational repression	33
Figure 2.1:	Types of RNAi effector	42
Figure 2.2:	Points of the RNAi pathway involved in determining silencing efficiency	47
Figure 2.3:	Influence of guide RNA structure on siRNA efficiency	48
Figure 2.4:	siRNA design that avoids off-target effects	49
Figure 2.5:	Mechanisms of immunostimulation with siRNA	50
Figure 2.6:	Advantages and disadvantages of siRNA synthesis methods	55
Figure 2.7:	Mechanism of ddRNAi compared to RNAi triggered by an siRNA	61
Figure 2.8:	Nucleonics multi-targeting Anti-HBV drug	64
Figure 3.1:	Considerations for a HT RNAi screen	73
Figure 3.2:	Structures of silencing reagents for RNAi screening	75
Figure 3.3:	Read-out methods for high throughput screens	82
Figure 3.4:	Method of ArteMice™ RNAi development	85
Figure 3.5:	Peer reviewed miRNA publications (2001-2006)	92
Figure 3.6:	Comparison of Mirus' <i>LabelIT</i> and enzymatically labelled samples	97
Figure 3.7:	Insert Comparison of effectiveness of different knockdown methods t figuretitlehere, with date where appropriate	100
Figure 4.1:	Chemical modifications of siRNAs increase stability and PK	108
Figure 4.2:	Ribo-T siRNAs from Nantech Pharmaceuticals	111
Figure 4.3:	Structure of a SNALP	114
Figure 4.4:	Mirus Bio's Dynamic PolyConjugates™ delivery system for siRNA	119
Figure 4.5:	Aptamer complexes for cell-type specific delivery of siRNAs	121
Figure 4.6:	Heavy-chain antibody fragment for delivery of siRNAs to cell surface receptors	122
Figure 4.7:	Targeted nanoparticles delivering siRNA	124
Figure 4.8:	Aptamer directed nanoparticle formulation of docetaxel	125
Figure 4.9:	Preparation of virosomes encapsulating siRNA	127
Figure 4.10:	Viral delivery of shRNA	128
Figure 5.1:	Development of AMD	136
Figure 5.2:	siRNA targeting VEGF reduces blood vessel growth in the cornea	138
Figure 5.3:	Visual Acuity data from Phase 1 studies of Sirna-027	142
Figure 5.4:	Possible targets for suppressing HIV replication	147
Figure 5.5:	siRNA mediated knockdown in Sirna's primate model of HCV chimeric infection	150
Figure 5.6:	Inhibition of three regions of the HCV genome by TT-033 for more than 2 months after a single administration to mice	151
Figure 5.7:	Down regulation of Hepatitis B Surface Antigen after intravenous delivery of Nucleonic's HBV candidate	153
Figure 6.1:	Rosetta Genomics' method for miRNA identification and validation	184
Figure 6.2:	Illustration of Exiqon's miRNA diagnostics	186
Figure 6.3:	Structure of an antagomir	188
Figure 6.4:	Asuragen's development of miRNA-based therapeutics	190
Figure 6.5:	<i>let-7</i> : tumor suppressor and future therapeutic	191
Figure 8.1:	RNAi market sectors	211
Figure 8.2:	Turnover of the RNAi market 2006-2015 (incl. drug discovery, development and marketing)	218

List of Tables

Table 1.1:	Failures of antisense drugs	27
Table 1.2:	Highly generalized comparison of siRNAs and miRNAs	34
Table 1.3:	Advantages of RNAi	36
Table 1.4:	Disadvantages of RNAi	37
Table 2.1:	Selection of publicly available siRNA design tools	43
Table 2.2:	Commercial siRNA design services	45
Table 2.3:	Suppliers of custom and predesigned siRNAs	56
Table 2.4:	Suppliers of siRNA pools and kits to create them	59
Table 2.5:	Comparison of plasmid-based vectors and synthetic siRNA for research purposes	62
Table 2.6:	Selection of plasmid vector products for siRNA generation	63
Table 2.7:	Suppliers of kits for generating siRNA expression cassettes by PCR	65
Table 2.8:	Advantages and disadvantages of different viral and non-viral expression vectors	66
Table 2.9:	Selection of suppliers of viral vectors for gene silencing	67
Table 3.1:	Suppliers of siRNA libraries for HT screening (3.1)	76
Table 3.2:	Commercial availability and coverage of shRNA libraries	78
Table 3.3:	Summary of the pros and cons of the different silencing reagents for RNAi screening	80
Table 3.4:	Advantages and disadvantages of RNAi knock-down <i>in vivo</i>	86
Table 3.5:	Key databases and algorithms for miRNA target prediction	93
Table 3.6:	Suppliers of miRNA mimics and inhibitors	99
Table 4.1:	Non-viral methods for siRNA delivery	112
Table 5.1:	RNAi therapeutics in, or close to, clinical development	135
Table 5.2:	RNAi therapeutic targets in neurological disease	161
Table 5.3:	Atugen's pipeline of anticancer therapeutics	167
Table 6.1:	miRNA expression profiling in human cancers	183
Table 7.1:	Seminal patents in RNAi	197
Table 7.2:	Issues in <i>Tuschl I vs Tuschl II</i>	198
Table 7.3:	Licensees of Alnylam's RNAi patents	200
Table 7.4:	Chemical modifications – Sirna's patent portfolio	202
Table 7.5:	Therapeutic targets for RNAi – Sirna's patent portfolio	204
Table 8.1:	Companies involved in RNAi technologies, A-B	214
Table 8.2:	Companies involved in RNAi technologies, C-G	215
Table 8.3:	Companies involved in RNAi technologies, I-O	216
Table 8.4:	Companies involved in RNAi technologies, P-Z	217
Table 8.5:	Sales forecast for total RNAi market, 2006-2015 (reagents, delivery vehicles, therapeutic products)	218
Table 8.6:	Sales forecasts for RNAi suppliers: synthesis and reagents, 2006 – 2015	222
Table 8.7:	Pharma's alliances for RNAi in R&D	223
Table 8.8:	Sales forecasts for delivery of RNAi therapeutics, 2006-2015	225
Table 8.9:	Costs associated with RNAi therapeutic development	226
Table 8.10:	Potential value of therapy areas targeted by RNAi therapeutics, 2005 & 2010	227
Table 8.11:	Sales forecasts for RNAi therapeutic drugs launched 2010-2015	229
Table 8.12:	Pharma's alliances for RNAi in R&D	230