

TABLE OF CONTENTS

0. Executive Summary	14
1. Technologies for suppressing gene function	16
Introduction	16
DNA transcription.....	16
RNA	16
<i>Non-coding RNA</i>	<i>16</i>
<i>RNA research and potential applications.....</i>	<i>17</i>
<i>Role of RNA in regulation of the dihydrofolate reductase gene</i>	<i>18</i>
Gene regulation	18
Post-transcriptional regulation of gene expression.....	19
Alternative RNA splicing.....	20
Technologies for gene suppression	20
Antisense oligonucleotides	20
Transcription factor decoys	21
Aptamers.....	21
Ribozymes	22
Aptazymes.....	22
RNA aptamers vs allosteric ribozymes	23
RNA Lasso.....	23
Peptide nucleic acid.....	23
PNA-DNA chimeras.....	24
Locked nucleic acid	24
Gene silencing	24
Post-transcriptional gene silencing	25
TargeTron™ technology for gene knockout	25
Definitions and terminology of RNAi	25
RNAi mechanisms	26
Non-promoter-associated small RNAs.....	28
Piwi-interacting RNAs in germ cell development	29
Relation of RNAi to junk DNA.....	29
RNA editing and RNAi	30
Historical landmarks in the development of RNAi	30
2. RNAi Technologies	32
Introduction	32
Comparison of antisense and RNAi	32
Advantages of antisense over siRNAs	32
Advantages of siRNAs over antisense	33
RNA aptamers vs siRNA	33
RNA Lassos versus siRNA.....	33
Concluding remarks on antisense vs RNAi	34
ssRNAi	34
Antisense vs DNP-ssRNA and DNP-siRNA	34
LNA and RNAi.....	35
LNA for gene suppression.....	35
Comparison of LNA and RNAi.....	36
Use of siLNA to improve siRNA.....	36
RNAi versus small molecules	36
RNAi in vivo	36
Cre-regulated RNAi in vivo	37
RNAi kits.....	37
ShortCut™ RNAi Kit.....	37
HiScribe™ RNAi Transcription Kit	38
pSUPER RNAi system.....	38
Si2 Silencing Duplex.....	39
Techniques for measuring RNAi-induced gene silencing	39
Application of PCR in RNAi.....	39
<i>Real-time quantitative PCR.....</i>	<i>40</i>
<i>Assessment of the silencing effect of siRNA by RT-PCR</i>	<i>40</i>
<i>Fluorescence resonance energy transfer probe for RNA interactions</i>	<i>41</i>
Bioinformatics tools for design of siRNAs	41
Random siRNA design.....	41
Rational siRNA design.....	42
<i>The concept of pooling siRNAs</i>	<i>43</i>
<i>Criteria for rational siRNA design</i>	<i>43</i>
<i>BLOCK-iT RNAi Designer</i>	<i>43</i>

<i>QIAGEN's 2-for-Silencing siRNA Duplexes</i>	44
<i>Designing vector-based siRNA</i>	44
<i>iRNAChek for designing siRNA</i>	44
<i>TROD: T7 RNAi Oligo Designer</i>	44
<i>siDirect: siRNA design software</i>	45
Prediction of efficacy of siRNAs	45
<i>Algorithms for prediction of siRNA efficacy</i>	45
<i>siRNA databases</i>	45
Production of siRNAs	46
Chemical synthesis of short oligonucleotides	46
In vitro transcription	46
Generation of siRNAs in vivo	47
UsiRNAs.....	47
siRNA:DNA hybrid molecules	48
Chemical modifications of siRNAs	48
<i>Sugar modifications of siRNA</i>	48
<i>Phosphate linkage modifications of siRNA</i>	48
<i>Modifications to the siRNA overhangs</i>	49
<i>Modifications to the duplex architecture</i>	49
<i>Applications of chemical modification of siRNAs</i>	49
Synthetic RNAs vs siRNAs	50
Specificity of siRNAs	50
Asymmetric interfering RNA	51
Genome-wide data sets for the production of esiRNAs	51
ddRNAi for inducing RNAi	51
ddRNAi technology	51
Advantages of ddRNAi over siRNA	52
Short hairpin RNAs	53
siRNA versus shRNA	53
Circular interfering RNA	54
Expressed interfering RNA	55
RNA-induced transcriptional silencing complex	55
Inhibition of gene expression by antigene RNA	56
RNAi vs mRNA modulation by small molecular weight compounds	56
3. MicroRNA	58
Introduction	58
miRNA and RISC	60
Role of the microprocessor complex in miRNA	60
miRNAs compared to siRNAs	61
miRNA and stem cells	62
Influence of miRNA on stem cell formation and maintenance	62
Role of miRNAs in gene regulation during stem cell differentiation.....	62
miRNA databases	63
Sanger miRBase miRNA sequence database	63
Mapping miRNA genes.....	63
A database of ultraconserved sequences and miRNA function	64
A database for miRNA deregulation in human disease.....	64
An database of miRNA-target interactions	65
Role of miRNA in gene regulation	65
Control of gene expression by miRNA	66
<i>miRNA-mediated translational repression involving Piwi</i>	66
<i>Transcriptional regulators of ESCs control of miRNA gene expression</i>	66
Mechanism of miRNAs-induced silencing of gene expression.....	66
miRNA diagnostics	67
Biochemical approach to identification of miRNA	67
Computational approaches for the identification of miRNAs	68
LNA probes for exploring miRNA	68
Microarrays for analysis of miRNA gene expression	68
<i>Microarrays vs quantitative PCR for measuring miRNAs</i>	69
miRNAs as biomarkers of hepatotoxicity	69
Modification of in situ hybridization for detection of miRNAs	70
Nuclease Protection Assay to measure miRNA expression.....	70
Real-time PCR for expression profiling of miRNAs.....	70
Targeting of miRNAs with antisense oligonucleotides	71
Silencing miRNAs by antagomirs.....	71
New tools for miRNA silencing	71
Use of HAPIscreen for identification of aptamers against pre-miRNAs	72
miRNA-regulated lentiviral vectors	72
miRNAs as drug targets	72
miRNAs as targets for antisense drugs.....	73

Challenges facing use of miRNAs as drug targets	73
Target specificity of miRNAs	74
Prediction of miRNA targets.....	74
Role of miRNA in human health and disease.....	75
Role of miRNAs in regulation of hematopoiesis.....	75
Role of miRNA depletion in tissue regeneration	75
Role of miRNA in regulation of aging	76
Role of miRNA in inflammation	76
Role of miRNAs in regulation of immune system	76
Role of miRNA in the cardiovascular system	77
Role of miRNAs in development of the cardiovascular system	77
Role of miRNAs in angiogenesis	77
Role of miRNAs in cardiac hypertrophy and failure	77
Role of miRNAs in conduction and rhythm disorders of the heart.....	78
Diagnostic and prognostic value of miRNAs in acute coronary syndrome	78
miRNA-based approaches for reduction of hypercholesterolemia	79
miRNA-based approach for restenosis following angioplasty	79
miRNA gene therapy for ischemic heart disease.....	79
miRNAs as therapeutic targets for cardiovascular diseases	80
Concluding remarks and future prospects of miRNA in the cardiovascular system.....	80
Role of miRNAs in the nervous system.....	80
miRNAs and addiction.....	81
miRNAs in neurodegenerative disorders	81
<i>miRNAs as biomarkers of Alzheimer's disease.....</i>	<i>82</i>
<i>miRNAs in Huntington's disease.....</i>	<i>82</i>
<i>miRNA malfunction in spinal motor neuron disease.....</i>	<i>82</i>
<i>miRNAs and retinal neurodegenerative disorders</i>	<i>83</i>
miRNA and schizophrenia.....	83
Role of miRNA in viral infections.....	83
Role of miRNA in HSV-1 latency.....	83
miRNA and autoimmune disorders.....	84
miRNA in rheumatoid arthritis	84
miRNA in systemic lupus erythematosus	84
miRNAs in gastrointestinal disorders.....	85
miRNA-based therapies for the irritable bowel syndrome	85
miRNA and skin disorders.....	85
Role of miRNA in inflammatory skin disorders.....	85
Role of miRNAs in cancer.....	85
miRNAs linked to the initiation and progression of cancer.....	85
Oncomirs	86
Linking miRNA sequences to cancer using RNA samples	87
Role of miRNAs in viral oncogenesis	87
miRNA genes in cancer	87
miRNAs interaction with p53	88
miRNAs, embryonic stem cells and cancer.....	88
miRNAs and cancer metastases	89
Role of miRNAs in cancer diagnosis	90
<i>Cancer miRNA signature</i>	<i>90</i>
<i>miRNA biomarkers in cancer.....</i>	<i>90</i>
<i>Diagnostic value of miRNA in cancer.....</i>	<i>91</i>
<i>Prognostic value of miRNA in cancer.....</i>	<i>91</i>
miRNAs as basis of cancer therapeutics	91
<i>Antisense oligonucleotides targeted to miRNA.....</i>	<i>91</i>
<i>Delivery of miRNA mimetics in Cancer</i>	<i>92</i>
<i>Role of miRNAs in adoptive immunotherapy of cancer</i>	<i>92</i>
<i>Restoration of tumor suppressor miRNA may inhibit cancer</i>	<i>92</i>
Role of miRNAs in various cancers.....	93
<i>miRNA and brain cancer.....</i>	<i>93</i>
<i>miRNA and breast cancer.....</i>	<i>94</i>
<i>miRNA and colorectal cancer</i>	<i>94</i>
<i>miRNA and gastrointestinal cancer</i>	<i>95</i>
<i>miRNA and hematological malignancies</i>	<i>95</i>
<i>miRNA and hepatocellular carcinoma</i>	<i>96</i>
<i>miRNA and lung cancer.....</i>	<i>97</i>
<i>miRNA and nasopharyngeal carcinoma</i>	<i>98</i>
<i>miRNA and ovarian cancer</i>	<i>98</i>
<i>miRNA and pancreatic cancer</i>	<i>99</i>
<i>miRNA and prostatic cancer.....</i>	<i>99</i>
<i>miRNA and thyroid cancer.....</i>	<i>100</i>
Future prospects of miRNA therapeutics.....	100
Companies involved in miRNA.....	101

4. Methods of delivery in RNAi	104
Introduction	104
Methods of delivery of oligonucleotides	104
Oral and rectal administration	105
Pulmonary administration	105
Targeted delivery to the CNS.....	105
<i>High flow microinfusion into the brain parenchyma</i>	106
Intracellular guidance by special techniques	106
Biochemical microinjection	107
Liposomes-mediated oligonucleotide delivery	107
Polyethylenimine-mediated oligonucleotide delivery	107
Delivery of TF Decoys.....	107
Biodegradable microparticles.....	108
<i>Microparticles</i>	108
<i>Nanoparticles</i>	108
Self-delivering rxRNA	108
siRNA delivery technologies	109
Local delivery of siRNA	110
In vivo delivery of siRNAs by synthetic vectors	110
Intracellular delivery of siRNAs	110
<i>Delivery of siRNAs with aptamer-siRNA chimeras</i>	111
<i>MPG-based delivery of siRNA</i>	111
<i>Nanoparticles for intracellular delivery of siRNA</i>	111
<i>Protamine-antibody fusion proteins for delivery of siRNA to cells</i>	111
<i>Protein transduction domains</i>	112
<i>Phosphorothioate stimulated cellular delivery of siRNA</i>	112
Targeted delivery of siRNAs by lipid-based technologies.....	112
<i>Delivery of siRNA-lipoplexes</i>	113
<i>Lipidoids for delivery of siRNAs</i>	113
<i>NeoLipid™ technology</i>	114
<i>siFECTamine™</i>	114
<i>Systemic in vivo delivery of lipophilic siRNAs</i>	114
<i>Systemic delivery of siRNAi by lipid nanoparticles</i>	114
<i>Challenges and future prospects of lipid-based siRNA delivery</i>	115
Electroporation	115
<i>Nucleofactor technology</i>	116
<i>Visualization of electrotransfer of siRNA at single cell level</i>	116
Intravascular delivery of siRNA	116
27mer siRNA duplexes for improved delivery and potency.....	117
TransIT-TKO®	117
DNA-based plasmids for delivery of siRNA	118
<i>Convergent transcription</i>	119
PCR cassettes expressing siRNAs	119
Genetically engineered bacteria for delivery of shRNA.....	119
Viral vectors for delivery of siRNA	119
<i>Adenoviral vectors</i>	119
<i>Adeno-associated virus vectors for shRNA expression</i>	120
<i>Baculovirus vector</i>	120
<i>Lentiviral vectors</i>	121
<i>Retroviral delivery of siRNA</i>	122
Transkingdom RNAi delivery by genetically engineered bacteria.....	122
Delivery of siRNA without a vector	122
Cell-penetrating peptides for delivery of siRNAs	123
Role of nanobiotechnology in siRNA delivery.....	123
<i>Chitosan-coated nanoparticles for siRNA delivery</i>	123
<i>Cyclodextrin nanoparticles</i>	124
<i>Delivery of gold nanorod-siRNA nanoplex to dopaminergic neurons</i>	124
<i>Lipidic aminoglycoside as siRNA nanocarrier</i>	124
<i>Lipid nanoparticles-mediated siRNA delivery</i>	124
<i>Nanosize liposomes for delivery of siRNA</i>	125
<i>PAMAM dendrimers for siRNA delivery</i>	125
<i>Polyethylenimine nanoparticles for siRNA delivery</i>	126
<i>Polycation-based nanoparticles for siRNA delivery</i>	126
<i>Quantum dots to monitor siRNA delivery</i>	127
Targeted delivery of siRNAs to specific organs	127
<i>siRNA delivery to the CNS</i>	127
<i>siRNA delivery to the liver</i>	128
<i>siRNA delivery to the lungs</i>	129
Control of RNAi and siRNA levels	129
siRNA pharmacokinetics in mammalian cells	129
Mathematical modeling for determining the dosing schedule of siRNA.....	130

Assessing siRNA pharmacodynamics in animal models.....	130
Research on siRNA delivery funded by the NIH.....	131
Companies involved in delivery technologies for siRNA	131
5. RNAi in Research	134
Introduction	134
Basic RNAi research	134
<i>Antiviral role of RNAi in animal cells</i>	<i>134</i>
<i>Combination of siRNA with green fluorescent protein</i>	<i>134</i>
<i>Detection of cancer mutations</i>	<i>135</i>
<i>Genes and lifespan.....</i>	<i>135</i>
<i>Inducible and reversible RNAi.....</i>	<i>135</i>
<i>Loss-of-function genetic screens</i>	<i>135</i>
<i>Profiling small RNAs</i>	<i>136</i>
<i>RNAi for research in neuroscience</i>	<i>136</i>
<i>RNAi and environmental research</i>	<i>137</i>
<i>Silencing snoRNA genes.....</i>	<i>137</i>
<i>Study of signaling pathways.....</i>	<i>137</i>
<i>Transgenic RNAi.....</i>	<i>138</i>
<i>Use of RNAi to study insulin action</i>	<i>138</i>
Applied RNAi research	138
RNAi for gene expression studies	138
Microarrays for measuring gene expression in RNAi.....	138
RNAi for functional genomic analysis	139
<i>RNAi studies on C. elegans.....</i>	<i>139</i>
<i>RNAi studies on Drosophila.....</i>	<i>140</i>
<i>RNAi in planaria</i>	<i>141</i>
Testing the specificity of RNAi.....	141
Tissue-specific RNAi	141
siRNA-mediated gene silencing	142
RNAi libraries	142
<i>Universal plasmid siRNA library.....</i>	<i>143</i>
<i>pDual library using plasmid vector</i>	<i>143</i>
<i>pHippy plasmid vector library</i>	<i>144</i>
<i>siRNA library including miRNAs</i>	<i>144</i>
<i>siRNA libraries using pRetroSuper vector</i>	<i>144</i>
<i>siRNA produced by enzymatic engineering of DNA.....</i>	<i>144</i>
<i>shRNA libraries</i>	<i>145</i>
<i>Enzymatic production of RNAi library</i>	<i>146</i>
RNAi and alternative splicing	146
RNAi in animal development.....	146
<i>RNAi for creating transgenic animals</i>	<i>147</i>
<i>RNAi for creating models of neurological disorders</i>	<i>147</i>
Research support for RNAi in US.....	148
RNAi for toxicogenomics	148
Role of RNAi in the US biodefense research	148
The RNAi Consortium	148
Research support for RNAi in Europe.....	149
European Union for RNA Interference Technology	149
Research support of RNAi.....	150
Role of RNAi in MitoCheck project	150
RNAi Global Initiative	151
SIROCCO project.....	152
6. RNAi in drug discovery	154
Basis of RNAi for drug discovery	154
RNAi for identification of genes as therapeutic targets	154
Role of siRNAs in drug target identification	155
<i>Use of a genome-wide, siRNA library for drug discovery.....</i>	<i>155</i>
<i>Use of arrayed adenoviral siRNA libraries for drug discovery.....</i>	<i>155</i>
RNAi as a tool for assay development.....	156
Targeting human kinases with an siRNAi library	156
Challenges of drug discovery with RNAi	156
Express Track SM siRNA Drug Discovery Program.....	157
Genome-wide siRNA screens in mammalian cells	157
<i>PhenomicIDTM</i>	<i>157</i>
Natural antisense and ncRNA as drug targets	158
RNAi for target validation.....	158
<i>Delivering siRNA for target validation in vivo</i>	<i>158</i>
<i>Off-target effects of siRNA-mediated gene silencing</i>	<i>160</i>
<i>Bioinformatic approach to off-target effects.....</i>	<i>161</i>

<i>Validation of oncology targets discovered through RNAi screens</i>	161
<i>Selection of siRNA versus shRNA for target validation</i>	162
Application of RNAi to the druggable genome	162
Application of siRNA during preclinical drug development.....	162
siRNAs vs small molecules as drugs.....	163
siRNAs vs antisense drugs.....	163
RNAi technology in plants for drug discovery and development.....	164
<i>Application of RNAi to poppy plant as source of new drugs</i>	164
7. Therapeutic applications of RNAi.....	166
Introduction	166
Potential of RNAi-based therapies	167
In vitro applications of siRNA	167
In vivo applications of RNAi.....	168
RNAi and cell therapy	168
Gene inactivation to study hESCs.....	169
RNAi and stem cells	169
Cell therapy for immune disorders	170
RNAi gene therapy	170
Drug-inducible systems for control of gene expression.....	170
Potential side effects of RNAi gene therapy.....	171
Systemic delivery of siRNAs	171
In vivo RNAi therapeutic efficacy in animal models of human diseases.....	172
Virus infections.....	172
RNAi approaches to viral infections.....	173
<i>Delivery of siRNAs in viral infections</i>	174
RNAi applications in HIV	174
<i>A multiple shRNA approach for silencing of HIV-1</i>	175
<i>Anti-HIV shRNA for AIDS lymphoma</i>	175
<i>Aptamer-mediated delivery of anti-HIV siRNAs</i>	175
<i>Bispecific siRNA constructs</i>	175
<i>Role of the nef gene during HIV-1 infection and RNAi</i>	176
<i>siRNA-directed inhibition of HIV-1 infection</i>	176
<i>Synergistic effect of snRNA and siRNA</i>	177
<i>Targeting CXCR4 with siRNAs</i>	177
<i>Targeting CCR5 with siRNAs</i>	177
<i>Concluding remarks on RNAi approach to HIV/AIDS</i>	178
Influenza	178
<i>Inhibition of influenza virus by siRNAs</i>	179
<i>Delivery of siRNA in influenza</i>	180
<i>Challenges and future prospects of siRNAs for influenza</i>	180
Respiratory syncytial and parainfluenza viruses	181
Coronavirus/severe acute respiratory syndrome	182
Herpes simplex virus 2	182
Hepatitis B	182
Hepatitis C virus	183
Cytomegalovirus.....	185
Ebola virus.....	185
siRNA vs antisense oligonucleotides for viral infections	185
siRNA against methicillin-resistant <i>S. aureus</i>	186
RNAi-based rational approach to antimalarial drug discovery	186
Inhibiting the growth of malarial parasite by heme-binding DNA aptamers.....	186
siRNA-based antimalarial therapeutics.....	187
RNAi applications in oncology.....	187
Allele-specific inhibition	188
Drug delivery issues in managing cancer by RNAi approach.....	188
Inhibition of oncogenes.....	189
Modification of alternative splicing in cancer	190
Onconase.....	190
Overcoming drug resistance in cancer	191
<i>Targeting fusion proteins in cancer</i>	192
<i>Increasing chemosensitivity by RNAi</i>	192
RNAi approach to study TRAIL	192
RNAi-based logic circuit for identification of specific cancer cells.....	193
siRNAs for anticancer drug discovery	193
siRNAs for inducing cancer immunity	194
siRNAs for inhibition of angiogenesis	194
siRNA targeting the R2 subunit of ribonucleotide reductase	195
siRNA for cancer chemoprevention	195
siHybrids vs siRNAs as anticancer agents	195
Nanobiotechnology-based delivery of siRNAs	196

<i>Lipid nanoparticle-based delivery of anticancer siRNAs</i>	196
<i>Minicells for targeted delivery of nanoscale anticancer therapeutics</i>	196
<i>Nanoimmunoliposome-based system for targeted delivery of siRNA</i>	197
<i>Polymer nanoparticles for targeted delivery of anticancer siRNA</i>	197
<i>RNA nanotechnology for delivery of cancer therapeutics</i>	198
<i>Targeted delivery of a nanoparticle-siRNA complex in cancer patients</i>	198
RNAi-based treatment of various cancer types.....	199
<i>RNAi-based therapy of brain cancer</i>	199
<i>RNAi in breast cancer</i>	200
<i>RNAi for enhancing hyperthermia/chemotherapy in cervical cancer</i>	201
<i>RNAi and colorectal cancer</i>	201
<i>RNAi and Ewing's sarcoma</i>	202
<i>RNAi and leukemias</i>	202
<i>RNAi and lung cancer</i>	203
<i>RNAi and melanoma</i>	203
<i>RNAi and pancreatic cancer</i>	204
<i>RNAi and prostate cancer</i>	204
Genetic disorders	204
RNAi for skin disorders	205
Experimental studies for RNAi applications in skin disorders	205
Clinical applications of RNAi in skin disorders	206
Pachyonychia congenita	206
Neurological disorders	206
RNAi for neurodegenerative disorders	207
<i>Alzheimer's disease</i>	208
<i>Parkinson's disease</i>	208
<i>Amyotrophic lateral sclerosis</i>	209
<i>Prion diseases</i>	210
Polyglutamine-induced neurodegeneration	210
<i>Fragile X syndrome and RNAi</i>	211
<i>RNAi-based therapy for Huntington's disease</i>	211
Combination of RNAi and gene therapy to prevent neurodegenerative disease.....	212
Role of RNAi in pain therapy.....	213
Role of RNAi in repair of spinal cord injury	213
Role of RNAi in treatment of multiple sclerosis	214
siRNA for Duchenne muscular dystrophy	214
siRNA for dystonia.....	214
RNAi in ophthalmology	215
Age related macular degeneration.....	215
<i>Current treatment of AMD</i>	215
<i>RNAi-based treatments for AMD</i>	216
Diabetic retinopathy	217
Retinitis pigmentosa.....	218
RNAi and metabolic disorders	218
RNAi and obesity	219
<i>Genes and regulation of body fat</i>	219
RNAi and diabetes.....	219
<i>Regulation of insulin secretion by a miRNA</i>	219
<i>RNAi for study of genes in animal models of diabetes</i>	220
<i>RNAi for drug discovery in diabetes</i>	220
<i>RNAi for treating liver dysfunction in diabetes</i>	221
<i>siRNAs for study of glucose transporter</i>	221
<i>siRNAs for targeting adipose inflammation in diabetes and obesity</i>	222
RNAi in hematology	222
Stem cell-based gene therapy and RNAi for sickle cell disease	222
RNAi and disorders of the immune system	223
siRNA applications in immunology	223
Use of RNAi in transplantation	224
RNAi for cardiovascular disorders	224
RNAi for hypercholesterolemia	225
siRNA targeting NADPH oxidase in cardiovascular diseases.....	225
siRNA for study and treatment of ischemia-reperfusion injury	226
RNAi in respiratory disorders	226
siRNA for cystic fibrosis	226
siRNA for asthma	227
RNAi for musculoskeletal disorders	227
RNAi for rheumatoid arthritis.....	227
RNAi for bone disorders	228
<i>RNAi for treatment of osteoporosis</i>	228
Research relevant to RNAi-based therapies at academic institutes	229
Laboratory of RNA Molecular Biology, The Rockefeller University	229

RNAi Center, La Jolla Institute for Allergy & Immunology	229
Clinical trials of RNAi-based therapies	230
Improving efficacy of siRNAs for clinical trials by improved delivery	231
Role of RNAi in development of personalized medicine.....	231
Future prospects of RNAi	232
Challenges for the development of RNAi-based therapeutics.....	232
8. Safety, regulatory and patent issues	234
Introduction	234
Limitations and drawbacks of RNAi	234
Adverse effects of RNAi	234
Effect of siRNAs on interferon response	235
Detection of interferon response	235
Prevention of the interferon response in RNAi	236
Overcoming the innate immune response to siRNAs	236
Toxicity associated with RNAi	237
Selection of siRNAs to improve specificity and efficacy.....	237
Regulatory issues relevant to RNAi.....	237
RNAi patents.....	238
Companies with strong patent position	238
<i>Alnylam</i>	238
<i>Benitec</i>	241
<i>Intradigm</i>	241
<i>Quark Pharmaceuticals</i>	241
<i>Sirna Therapeutics</i>	242
9. Markets for RNAi Technologies	244
Introduction	244
Current and future market potential for RNAi technologies	244
RNAi reagents	245
siRNA markets.....	245
RNAi-based drug discovery and target validation	245
RNAi-based development of therapeutics	245
RNAi market potential according to therapeutic areas.....	246
Market for viral infections	246
Market for cancer.....	247
Market for age related macular degeneration.....	247
Unmet needs in RNAi	247
Strategies for marketing RNAi	248
Choosing optimal indications	248
Strategies according to the trends in healthcare in the next decade	249
Concluding remarks	250
10. Companies involved in RNAi Technologies	252
Introduction	252
Major players in RNAi	255
Profiles of companies.....	256
Collaborations.....	444
11. References.....	452

Tables

Table 1-1: Classification of small RNA molecules.....	26
Table 1-2: Mechanisms of small RNAs involved in gene silencing	27
Table 1-3: Historical landmarks in the evolution of RNAi	30
Table 2-1: RNAi versus small molecules.....	36
Table 2-2: Providers of software for siRNA design	42
Table 2-3: Methods for the production of siRNAs	46
Table 2-4: Advantages and limitations of methods of shRNA-derived siRNA knockdown	54
Table 2-5: Comparison of eiRNA with siRNA	55
Table 3-1: Methods for miRNA target prediction	74
Table 3-2: miRNA expression in neurodegenerative diseases.....	81
Table 3-3: Dysregulation of miRNA expression in epithelial cancers.....	86
Table 3-4: Companies involved in miRNA diagnostics and therapeutics.....	101
Table 4-1: Methods of delivery of oligonucleotides	104
Table 4-2: Methods of delivery of siRNA	109
Table 4-3: Companies developing siRNA delivery technologies.....	132
Table 5-1: RNAi libraries.....	142

Table 6-1: Delivery of siRNAs in vivo for target validation	159
Table 6-2: Selection of siRNA versus shRNA for target validation	162
Table 7-1: RNAi-based therapeutic approaches.....	167
Table 7-2: In vivo RNAi therapeutic efficacy in animal models of human diseases	172
Table 7-3: Inhibition of viral replication by RNAi	173
Table 7-4: Cancer-associated genes that can be targeted by RNAi	189
Table 7-5: Neurological disorders that have been studied by using RNAi	207
Table 7-6: Clinical trials of RNAi-based therapeutics.....	230
Table 9-1: RNAi markets according to technologies and reagents 2011-2021	244
Table 9-2: Markets for RNAi therapy for selected diseases: years 2011-2021	246
Table 10-1: RNAi reagent, technology and service companies	252
Table 10-2: Pharmaceutical companies using RNAi for drug discovery and development.....	253
Table 10-3: Biotechnology companies using RNAi for drug discovery and development.....	254
Table 10-4: Companies developing RNAi-based therapeutic products	255
Table 10-5: Major players in RNAi.....	255
Table 10-6: RNAi products of Benitec	276
Table 10-7: Proprietary reagents of ImuThes	331
Table 10-8: Product pipeline of Silence Therapeutics	413
Table 10-9: Collaborations in RNAi technologies	444

Figures

Figure 1-1: Relationship of DNA, RNA and protein in the cell	19
Figure 1-2: Schematic of suppression of gene expression by RNAi	27
Figure 2-1: Overview of ShortCut RNAi Kit.....	38
Figure 2-2: Gene silencing by RNAi induced with ddRNAi	52
Figure 3-1: A schematic miRNA pathway	58
Figure 3-2: Molecular mechanisms of miRNA generation.....	59
Figure 7-1: Targeting disease by RNAi.....	166
Figure 7-2: Role of RNAi in personalized medicine.....	232
Figure 8-1: Problems with use of synthetic siRNAs and measures to prevent them.....	235
Figure 9-1: Unmet needs in RNAi technologies	248