

# **Gene Therapy**

## **Part I: Technologies & Markets**

**by**

**Prof. K. K. Jain**  
MD, FRACS, FFPM  
**Jain PharmaBiotech**  
**Basel, Switzerland**

**November 2021**

**A Jain PharmaBiotech Report**

## A U T H O R ' S   B I O G R A P H Y

Professor K. K. Jain is a neurologist/neurosurgeon with specialist qualifications including Fellowships of the Royal Colleges of Surgeons in Australia and Canada. He has trained, practiced and held academic positions in several countries including Switzerland, Germany Canada and USA. After his retirement from neurosurgery, he is working in the biotechnology/biopharmaceuticals industry and is a Fellow of the Faculty of Pharmaceutical Medicine of the Royal College of Physicians of UK. Currently, he is a consultant at Jain PharmaBiotech.

Prof. Jain's 492 publications include 35 books (6 as editor+ 29 as author) and 50 special reports, which have covered important areas in biotechnology, gene therapy and biopharmaceuticals, biomarkers: proteomics, molecular diagnostics, nanobiotechnology, and personalized medicine. Contributions to MedLink, an accredited continuing education program for neurologists, include 172 articles out of a total of 1200 articles by 450 authors. These articles are updated on a yearly basis. Prof. Jain's earlier books were the first in the areas covered: "Handbook of Laser Neurosurgery" (Charles C. Thomas, Springfield, Ill, 1983) and "Textbook of Hyperbaric Medicine" (1st ed in 1990 and 6th ed by Springer, 2017). Recent books include "Handbook of Nanomedicine" (Springer 2008, Chinese edition by Peking University Press 2011, 3rd ed 2017), "Textbook of Personalized Medicine" (Springer 2009; Japanese ed 2012; 2nd ed Springer 2015, 3rd ed 2021), "Handbook of Biomarkers" (Springer 2010; Chinese ed, Chemical Industry Press 2016, 2nd ed 2017), "Drug-induced Neurological Disorders", 4th ed (Springer 2021), "Handbook of Neuroprotection" (Springer 2011, 2nd ed 2019), "Applications of Biotechnology in Cardiovascular Therapeutics" (Springer 2011), "Applications of Biotechnology in Neurology" (Springer 2013), and "Applications of Biotechnology in Oncology" (Springer 2014). He has also edited 3 editions of "Drug Delivery System" (Springer 2008, 20012 and 2020) and "Applied Neurogenomics" (Springer 2015). Lectures on personalized medicine given at Kazakh National Medical University, Kazakhstan were translated into Russian and published as a book "Essentials of Personalized Medicine" (LITERRA Publishing House, Moscow, 2019). He is currently writing the "Handbook of Alzheimer Disease" to be published by Springer in 2022.

## A B O U T   T H I S   R E P O R T

Prof. Jain wrote the first commercial report on Gene Therapy in 1995, which was published by PJB Publication, London. This was followed by reports on Gene Therapy Vectors and Cancer Gene Therapy (1996). A report on Gene Therapy of Neurological Disorders was published by Decision Resources in 1997. In 1998, Prof. Jain wrote a Textbook of Gene Therapy, which was the first book on this subject to be translated into the Chinese language in 2000. A book on gene therapy companies was published in 2000 by John Wiley & Sons and continued to be updated online at Wiley's web site until 2003. In 2004, when the copyright reverted to Prof. Jain, it became part II of the Gene Therapy report. This report supercedes and updates all the previous books as well as reports.

**November 2021**  
**Copyright ©2021 by**

**Jain PharmaBiotech**  
Bläsiring 7  
CH-4057 Basel  
Switzerland

**Tel & Fax:** +4161-6924461  
**Email:** info@pharmabiotech.ch  
**Web site:** http://pharmabiotech.ch/

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, or otherwise without the prior written permission of the Publisher. This report may not be lent, resold, or otherwise traded in any manner without the consent of the Publisher. While all reasonable steps have been taken to ensure the accuracy of the information presented, the Publisher cannot accept responsibility for inadvertent errors or omissions.

# T A B L E   O F   C O N T E N T S

<b>0. Executive Summary .....</b>	<b>21</b>
<b>1. Introduction .....</b>	<b>23</b>
<b>Definitions .....</b>	<b>23</b>
<b>Historical evolution of gene therapy .....</b>	<b>23</b>
<b>Relation of gene therapy to other biotechnologies.....</b>	<b>25</b>
<b>Molecular biological basics for gene therapy .....</b>	<b>26</b>
Genome.....	26
DNA .....	27
RNA .....	27
<i>Transcriptome .....</i>	28
<i>RNA splicing .....</i>	28
Genes.....	29
<i>Silent gene mutations.....</i>	29
Gene regulation .....	30
Gene expression .....	30
ENCODE .....	31
Chromosomes .....	32
Telomeres.....	33
Mitochondria .....	33
Proteins .....	34
<b>2. Gene Therapy Technologies .....</b>	<b>35</b>
<b>Classification of gene therapy techniques.....</b>	<b>35</b>
<b>Ex vivo and in vivo gene therapy .....</b>	<b>36</b>
Ex vivo gene therapy.....	37
In vivo gene therapy .....	37
<b>Physical methods of gene transfer.....</b>	<b>37</b>
Electroporation .....	38
Applications of electroporation .....	38
<i>Clinical applications of electroporation .....</i>	39
<i>Advantages of electroporation .....</i>	40
<i>Limitations of electroporation .....</i>	40
Hydrodynamic .....	40
Microinjection .....	41
Particle bombardment .....	41
Ultrasound-mediated transfection .....	43
Molecular vibration.....	44
Application of pulsed magnetic field and superparamagnetic nanoparticles .....	44
Gene transfection using laser irradiation .....	44
Photochemical transfection.....	45
<b>Chemical methods of gene transfer .....</b>	<b>45</b>
<b>Vectors for gene therapy .....</b>	<b>45</b>
Basic considerations .....	45
Use of genes as pharmaceuticals .....	46
The ideal vector for gene therapy.....	46
Viral vectors .....	47
<i>Adenovirus vectors.....</i>	47
<i>Adeno-associated virus vectors.....</i>	49
<i>Alphavirus vectors.....</i>	52
<i>Baculovirus vectors .....</i>	53
<i>Foamy virus vectors .....</i>	53
<i>Herpes simplex virus vectors .....</i>	54
<i>Lentiviral vectors .....</i>	56
<i>Multicistronic retroviral vectors .....</i>	57
<i>Retroviral vectors.....</i>	57
<i>Oncogenic potential of retroviral vectors .....</i>	59
<i>Quantification of viral vectors .....</i>	59
<i>Future of viral vectors .....</i>	60
<i>Companies using viral vectors .....</i>	60
Nonviral vectors for gene therapy .....	61
<i>Anionic lipid-DNA complexes .....</i>	62
<i>Cationic lipid-DNA complexes .....</i>	63
<i>Effects of shape of DNA molecules on delivery with nonviral vectors .....</i>	63
<i>Electrostatic modifications of surface to improve gene delivery .....</i>	63
<i>Liposomes for gene therapy .....</i>	63
<i>Liposome-nucleic acid complexes .....</i>	65

<i>Liposome-HVJ complex</i>	65
<i>Transposons DNA vectors</i>	66
<i>Polycation-DNA complexes (polyplexes)</i>	67
<i>Plasmid DNA vs minicircle DNA</i>	68
<i>Polymer vectors for gene therapy</i>	68
<i>Synthetic biology and DNA vectors</i>	69
<i>Synthetic peptide complexes</i>	69
<i>Future of nonviral vs viral vectors</i>	70
<b>Nanobiotechnology for gene therapy</b>	70
<i>Antisense nanoparticles</i>	70
<i>Dendrimers</i>	71
<i>Cochleates</i>	71
<i>Calcium phosphate nanoparticles as nonviral vectors</i>	71
<i>DNA nanoparticles as nonviral vectors</i>	71
<i>Gelatin nanoparticles for gene delivery</i>	72
<i>Lipid nanoparticles for nucleic acid delivery</i>	72
<i>Nanoparticles as nonviral vectors for gene therapy</i>	72
<i>Nanoparticles with virus-like function as gene therapy vectors</i>	73
<i>Nanobiolistics for nucleic acid delivery</i>	73
<i>Nonionic polymeric micelles for oral gene delivery</i>	74
<i>Silica nanoparticles as a nonviral vector for gene delivery</i>	74
<i>Virus-like particles as nonviral vector</i>	74
<i>Receptor-mediated endocytosis</i>	74
<i>Artificial viral vectors</i>	76
<i>Directed evolution of AAV to create efficient gene delivery vectors</i>	76
<i>Bacterial ghosts as DNA delivery systems</i>	76
<i>Bacteria plus nanoparticles for gene delivery into cells</i>	77
<i>Chromosome-based vectors for gene therapy</i>	77
<i>Mammalian artificial chromosomes (MACs)</i>	77
<i>Human artificial chromosomes (HACs)</i>	78
<i>ΦC31 integrase system</i>	79
<i>Companies using nonviral vectors</i>	79
<i>Concluding remarks about vectors</i>	80
<b>Gene repair and replacement</b>	81
<i>Gene repair by single-stranded oligonucleotides</i>	81
<i>History and current status of chimeroplasty</i>	81
<b>Genome editing</b>	82
<i>Gene editing by zinc finger nucleases</i>	82
<i>Gene editing by using short synthetic oligonucleotides</i>	83
<i>Genome engineering by using TALENs</i>	84
<i>Genome editing by homologous recombination</i>	84
<i>GeneRide technology for genome editing</i>	84
<i>Gene editing by homing endonucleases</i>	85
<i>ARCUS genome editing technology</i>	85
<i>Genome editing by using CRISPR system</i>	86
<i>A method for generating genome-wide gRNA libraries for CRISPR</i>	87
<i>CRISPR-genome organization system</i>	87
<i>CRISPR vs other gene editing tools</i>	88
<i>CRISPR-FRT</i>	88
<i>CRISPR for studying human embryogenesis</i>	89
<i>CRISPER system for creating animal models of human diseases</i>	89
<i>CRISPR/Cas9 technology for drug discovery</i>	89
<i>CRISPR/Cas9-mediated epigenetic editing system</i>	90
<i>Engineered miniature CRISPR-Cas system</i>	90
<i>Expanding the range of Cas9s</i>	91
<i>Inducible CRISPR/Cas9 for multiple gene targeting</i>	91
<i>Lipid nanoparticles for delivery of CRISPR-Cas9 for genome-editing</i>	91
<i>Nanocapsules for delivery of CRISPR-Cas9 for genome editing</i>	91
<i>Nano-CRISPR</i>	92
<i>Photoactivatable CRISPR-Cas9</i>	92
<i>Protein delivery and genome editing</i>	92
<i>RNA editing with CRISPR-Cas9</i>	93
<i>Potential clinical applications of CRISPR systems</i>	93
<i>Autosomal dominant retinitis pigmentosa</i>	94
<i>Clinical trial of CRISPR in human cancer</i>	94
<i>CRISPR for glioblastoma</i>	94
<i>Correction of a gene mutation in the human embryo</i>	94
<i>Glaucoma</i>	95
<i>PERV blockage by CRISPR to facilitate xenotransplantation</i>	95
<i>RNA editing with CRISPR</i>	96
<i>Use of CRISPR for β-hemoglobinopathies</i>	96

<i>Use of CRISPR to restore hereditary hearing loss .....</i>	97
<i>Complications and limitations of CRISPR/Cas9 technology.....</i>	98
<i>Companies developing CRISPR technology .....</i>	99
<i>Clinical studies of gene editing.....</i>	100
<i>Targeted genome editing by artificial nucleases .....</i>	101
<i>Ex vivo gene editing .....</i>	101
<i>DNA medicines based on gene editing .....</i>	101
<i>Comparison of gene editing with techniques for suppression of gene mutations .....</i>	102
<i>Future of gene editing .....</i>	102
<b>Base editing of the genome .....</b>	<b>103</b>
<i>Base editing by protein engineering.....</i>	103
<i>Adenine base editors for genomic DNA .....</i>	103
<b>Messenger RNA gene therapy .....</b>	<b>104</b>
<i>Spliceosome mediated RNA trans-splicing .....</i>	104
<b>Cell-mediated gene therapy .....</b>	<b>104</b>
<i>Fibroblasts .....</i>	105
<i>Skeletal muscle cells .....</i>	106
<i>Vascular smooth muscle cells .....</i>	106
<i>Keratinocytes .....</i>	106
<i>Hepatocytes .....</i>	107
<i>Lymphocytes.....</i>	107
<i>Regulating protein delivery by genetically encoded lymphocytes .....</i>	107
<i>Implantation of microencapsulated genetically modified cells .....</i>	107
<i>Stem cell gene therapy .....</i>	108
<i>Combination of gene therapy with therapeutic cloning .....</i>	109
<i>Expansion of transduced HSCs in vivo .....</i>	109
<i>Gene delivery to stem cells by artificial chromosome expression .....</i>	109
<i>Improving delivery of genes to stem cells .....</i>	109
<i>In utero gene therapy using stem cells .....</i>	110
<i>Lentiviral vectors for gene transfer to marrow stem cells .....</i>	110
<i>Linker based sperm-mediated gene transfer technology.....</i>	110
<i>Mesenchymal stem cells for gene therapy .....</i>	110
<i>Microporation for transfection of MSCs.....</i>	111
<i>Preventing immune rejection of transplanted stem cells.....</i>	111
<i>Therapeutic applications for hematopoietic stem cell gene transfer.....</i>	111
<i>Transdermal gene therapy for drug addiction .....</i>	111
<i>The future of hematopoietic stem cell gene therapy .....</i>	112
<b>Chimeric antigen receptor T cells in relation to gene therapy .....</b>	<b>112</b>
<i>Basics of CAR-T cell.....</i>	112
<i>Basis of anticancer effect of CAR-T cells.....</i>	112
<i>Smart T-cells.....</i>	113
<i>CAR-T cell manufacture .....</i>	113
<i>Companies developing CAR-T cell therapy .....</i>	113
<i>CAR NK cells derived from human iPSCs .....</i>	116
<i>Genome editing of CAR-T cells .....</i>	116
<i>Safety and efficacy of CAR-T cell therapy .....</i>	117
<i>Cytokine release syndrome .....</i>	117
<i>Precautions for CAR-T cell therapy .....</i>	118
<b>Role of genetically modified bacteria in gene therapy .....</b>	<b>119</b>
<b>Routes of administration for gene therapy .....</b>	<b>119</b>
<i>Direct injection of naked DNA .....</i>	120
<i>Intramuscular injection.....</i>	120
<i>Intravenous DNA injection .....</i>	120
<i>Intraarterial delivery .....</i>	121
<i>Companies with gene delivery devices/technologies .....</i>	121
<b>Targeted gene therapy.....</b>	<b>122</b>
<i>Targeted integration.....</i>	122
<i>Bacteriophage integrase system for site-specific gene delivery .....</i>	123
<i>Controlled-release delivery of DNA .....</i>	123
<b>Controlled gene therapy .....</b>	<b>124</b>
<i>Controlled delivery of genetic material .....</i>	124
<i>Controlled induction of gene expression .....</i>	124
<i>Drug-inducible systems for control of gene expression.....</i>	125
<i>Timed activation of gene therapy by a circuit based on signaling network .....</i>	125
<i>Small molecules for post-transcriptional regulation of gene expression .....</i>	125
<i>Light Activated Gene Therapy .....</i>	126
<i>Spatial control of gene expression via local hyperthermia.....</i>	126
<i>Companies with regulated /targeted gene therapy .....</i>	126
<b>Gene marking .....</b>	<b>127</b>
<b>Germline gene therapy .....</b>	<b>127</b>
<i>Potential applications of human germline genome modification .....</i>	128

Pros and cons of human germline genome modification .....	128
<b>Role of gene transfer in antibody therapy.....</b>	<b>130</b>
<b>Genetically engineered vaccines .....</b>	<b>130</b>
DNA vaccines .....	130
<i>DNA inoculation technology.....</i>	<i>131</i>
<i>Methods for enhancing the potency of DNA vaccines .....</i>	<i>131</i>
<i>Advantages of DNA vaccines .....</i>	<i>131</i>
Vaccine vectors .....	132
Challenges and limitations of genetically engineered vaccines.....	133
RNA vaccines.....	133
<i>Nonviral delivery of self-amplifying RNA vaccine .....</i>	<i>133</i>
Vaccines based on reverse genetics.....	134
<b>Technologies for gene suppression.....</b>	<b>134</b>
Antisense oligonucleotides .....	134
Transcription factor decoys .....	135
Aptamers.....	135
Ribozymes .....	136
Peptide nucleic acid.....	136
<i>Intracellular delivery of PNAs.....</i>	<i>136</i>
Locked nucleic acid .....	137
<i>Zorro-LNA.....</i>	<i>137</i>
Gene silencing .....	137
Post-transcriptional gene silencing .....	137
Definitions and terminology of RNAi .....	138
RNAi mechanisms .....	138
Inhibition of gene expression by antogene RNA .....	139
RNAi gene therapy .....	139
microRNA gene therapy .....	139
Viral vectors for RNAi and miRNA gene therapy .....	140
<b>Application of molecular diagnostic methods in gene therapy .....</b>	<b>141</b>
Use of PCR to study biodistribution of gene therapy vector .....	141
PCR for verification of the transcription of DNA .....	141
In situ PCR for direct quantification of gene transfer into cells .....	142
Detection of retroviruses by reverse transcriptase (RT)-PCR.....	142
Confirmation of viral vector integration .....	142
Monitoring of gene expression .....	142
<i>Monitoring of gene expression by green fluorescent protein.....</i>	<i>143</i>
<i>Monitoring in vivo gene expression by molecular imaging.....</i>	<i>143</i>
<i>Advantages of gene therapy compared with protein therapy.....</i>	<i>143</i>
<b>Gene therapy manufacture .....</b>	<b>144</b>
<b>Formulation, transport, and storage of materials for gene therapy .....</b>	<b>144</b>
<b>3. Clinical Applications of Gene Therapy .....</b>	<b>145</b>
<b>Introduction .....</b>	<b>145</b>
<b>Aging-related disorders .....</b>	<b>145</b>
Telomerase gene therapy in aging .....	145
Combination gene therapy for treatment of multiple age-related diseases.....	145
<b>Bone and joint disorders.....</b>	<b>146</b>
Bone fractures.....	146
Gene therapy for intervertebral disc degeneration .....	147
Spinal fusion .....	147
Osteogenesis imperfecta .....	148
Rheumatoid arthritis.....	148
<i>Local or systemic treatment .....</i>	<i>149</i>
<i>In vivo or ex vivo gene therapy of RA.....</i>	<i>149</i>
<i>Clinical trials of gene therapy for rheumatoid arthritis.....</i>	<i>150</i>
Gene therapy for osteoarthritis .....	151
<i>Gene therapy strategies for osteoarthritis .....</i>	<i>151</i>
<i>Clinical trials of gene therapy for osteoarthritis .....</i>	<i>152</i>
Sports injuries .....	152
<i>Repair of articular cartilage defects .....</i>	<i>153</i>
Regeneration and replacement of bone by gene therapy .....	153
<b>Bacterial infections .....</b>	<b>154</b>
Antisense approach to bacterial infections .....	154
<b>Dentistry.....</b>	<b>155</b>
Tissue engineering in dental implant defects .....	155
<b>Endocrine and metabolic disorders .....</b>	<b>155</b>
Introduction .....	155
Gene therapy of obesity.....	156
<i>AAV vector-mediated delivery of GDNF for obesity .....</i>	<i>156</i>
<i>Oligopeptide for targeted nonviral gene delivery to adipocytes.....</i>	<i>156</i>

<i>Viral vector-mediated leptin gene therapy</i> .....	157
Diabetes mellitus .....	157
Methods of gene therapy of diabetes mellitus .....	158
<i>Viral vector-mediated gene transfer in diabetes</i> .....	158
<i>Endogenous reprogramming of <math>\alpha</math> cells into <math>\beta</math> cells</i> .....	159
<i>Gene delivery with ultrasonic microbubble destruction technology</i> .....	159
<i>Genetically engineered cells for diabetes mellitus</i> .....	160
<i>Genetically altered liver cells</i> .....	160
<i>Genetically modified stem cells</i> .....	160
<i>Genetically engineered dendritic cells</i> .....	161
<i>Glucokinase and insulin co-expression</i> .....	161
<i>Leptin gene therapy</i> .....	161
<i>Concluding remarks about cell and gene therapy of diabetes</i> .....	161
Gene therapy of growth-hormone deficiency .....	162
<b>Gastrointestinal disorders.....</b>	<b>163</b>
Introduction .....	163
Methods of gene transfer to the gastrointestinal tract.....	163
<i>Direct delivery of genes</i> .....	163
<i>Naked plasmid DNA into the submucosa</i> .....	164
<i>Viral vectors</i> .....	164
<i>Receptor-mediated endocytosis</i> .....	164
Indications for gastrointestinal gene therapy .....	164
<i>Gene therapy for inflammatory disorders of the bowel</i> .....	165
Gene transfer to the salivary glands .....	165
<i>Potential clinical applications of salivary gene therapy</i> .....	166
<b>Hematology .....</b>	<b>166</b>
Hemophilias .....	167
<i>Gene therapy of hemophilia</i> .....	167
<i>Hemophilia A</i> .....	168
<i>Hemophilia B</i> .....	169
<i>Concluding remarks about gene therapy of hemophilias</i> .....	171
Hemoglobinopathies.....	172
<i>Gene therapy for <math>\beta</math>-thalassemia</i> .....	172
<i>Gene therapy for sickle cell disease</i> .....	174
<i>HSC-targeted gene therapy for SCD</i> .....	174
<i>Gene therapy and RNAi for SCD based on stem cells</i> .....	174
<i>Gene editing in sickle cell disease</i> .....	175
<i>Gene editing using ZFN as treatment for both SCD and <math>\beta</math>-thalassemia</i> .....	175
<i>Gene editing using CRISPR-Cas9 for both SCD and <math>\beta</math>-thalassemia</i> .....	176
<i>Gene therapy of Fanconi's anemia</i> .....	176
Acquired hematopoietic disorders.....	177
<i>Chronic acquired anemias</i> .....	177
<i>Neutropenia</i> .....	177
<i>Thrombocytopenia</i> .....	178
Concluding remarks about gene therapy of hemoglobinopathies .....	179
Companies involved in gene therapy of hematological disorders .....	179
<b>In utero/fetal gene therapy.....</b>	<b>179</b>
Fetal gene transfer techniques.....	180
Animal models of fetal gene therapy .....	181
Potential applications of fetal gene therapy .....	181
<i>Fetal gene therapy for cystic fibrosis</i> .....	181
<i>Fetal intestinal gene therapy</i> .....	182
<i>Gene therapy for fetal growth restriction</i> .....	182
<b>Hearing disorders .....</b>	<b>182</b>
Potential of gene therapy .....	183
Vectors for gene therapy of hearing disorders.....	183
Auditory hair cell replacement and hearing improvement by gene therapy .....	184
<b>Kidney diseases .....</b>	<b>184</b>
End-stage renal disease.....	184
Methods of gene delivery to the kidney .....	185
<i>Bone marrow stem cells for renal disease</i> .....	185
<i>Gene transfer into kidney by viral vectors</i> .....	186
<i>Gene transfer into the glomerulus by HVJ-liposome</i> .....	186
<i>Gene transfer to tubules with cationic polymer polyethylenimine</i> .....	186
<i>Liposome-mediated gene transfer into the tubules</i> .....	186
<i>Mesangial cell therapy</i> .....	187
<i>Non-viral gene transfer to the kidneys</i> .....	187
Gene therapy in animal experimental models of renal disease .....	187
Genetic manipulations of the embryonic kidney .....	188
Antisense intervention in glomerulonephritis .....	188
Gene therapy for renal fibrosis .....	188

Use of genetically engineered cells for uremia due to renal failure .....	189
Concluding remarks .....	189
<b>Liver disorders.....</b>	<b>189</b>
Techniques of gene delivery to liver .....	190
<i>Direct injection of DNA into liver</i> .....	191
<i>Local gene delivery by isolated organ perfusion</i> .....	191
<i>Liposome-mediated direct gene transfer</i> .....	191
<i>Retroviral vector for gene transfer to liver</i> .....	191
<i>Adenoviral vectors for gene transfer to liver</i> .....	191
<i>Receptor-mediated approach</i> .....	192
Cell therapy for liver disorders.....	192
<i>Transplantation of genetically modified hepatocytes</i> .....	192
<i>Genetically modified hematopoietic stem cells</i> .....	193
<i>Gene therapy by ex vivo transduced liver progenitor cells</i> .....	193
Gene therapy of genetic diseases affecting the liver .....	193
<i>Crigler-Najjar syndrome</i> .....	193
<i>Hereditary tyrosinemia type I (HT1)</i> .....	194
<i>Hereditary tyrosinemia type 3</i> .....	194
<i>Wilson's disease</i> .....	195
Gene therapy of acquired diseases affecting the liver .....	195
<i>Cirrhosis of liver</i> .....	195
<b>Ophthalmic disorders.....</b>	<b>195</b>
Introduction to gene therapy of ophthalmic disorders .....	195
Methods of gene therapy for ophthalmic disorders .....	196
<i>Delivery of gene therapy by intravitreal injection</i> .....	197
<i>DNA nanoparticles for nonviral gene transfer to the eye</i> .....	198
<i>Optogenetic gene therapy for blindness due to retinal degeneration</i> .....	198
Age-related macular degeneration .....	199
Inherited disorders of optic nerve affecting vision .....	200
<i>Leber hereditary optic neuropathy</i> .....	201
<i>Gene therapy for LHON</i> .....	201
Inherited retinal degenerations .....	202
<i>Choroideremia</i> .....	203
<i>Leber congenital amaurosis</i> .....	203
<i>Monogenic macular degeneration due to mutations in the BEST1 gene</i> .....	205
<i>Retinitis pigmentosa</i> .....	205
<i>Stargardt disease</i> .....	207
Other inherited disorders affecting vision .....	207
<i>Color blindness</i> .....	207
<i>Usher syndrome</i> .....	208
<i>X-linked retinoschisis</i> .....	209
Diabetic retinopathy .....	209
Future of gene therapy of inherited retinal degenerations .....	210
<i>Combining cell and gene therapies for retinal disorders</i> .....	210
Prevention of complications associated with eye surgery .....	211
<i>Prevention of proliferative retinopathy by gene therapy</i> .....	211
<i>Posterior capsule opacification after cataract surgery</i> .....	211
Autoimmune uveitis .....	211
Retinal ischemic injury .....	211
Corneal disorders .....	212
Glaucoma .....	212
Corneal scarring .....	213
Companies developing gene therapy for eye disorders .....	213
<b>Organ transplantation.....</b>	<b>213</b>
Introduction .....	213
DNA vaccines for transplantation .....	214
Gene therapy for prolonging allograft survival .....	214
<i>Genetically modified Tregs expressing CAR for preventing GVHD</i> .....	214
Gene therapy in lung transplantation .....	215
Role of gene therapy in liver transplantation .....	215
Gene therapy in kidney transplantation .....	215
Veto cells and transplant tolerance .....	216
<b>Pulmonary disorders.....</b>	<b>216</b>
Techniques of gene delivery to the lungs .....	216
<i>Adenoviral vectors</i> .....	217
<i>Nonviral vectors</i> .....	218
<i>Aerosolization as an aid to gene transfer to lungs</i> .....	218
Cystic fibrosis .....	218
<i>Genetics and clinical features</i> .....	218
<i>Gene therapy for CF</i> .....	219
<i>CFTR gene transfer in CF</i> .....	219

<i>Concluding remarks about gene therapy of CF</i> .....	220
Miscellaneous pulmonary disorders .....	221
<i>Gene therapy for pulmonary arterial hypertension</i> .....	221
<i>Gene therapy for bleomycin-induced pulmonary fibrosis</i> .....	222
<i>Gene therapy of emphysema due to α1-antitrypsin deficiency</i> .....	222
<i>Gene therapy for asthma</i> .....	222
<i>Gene therapy for adult respiratory distress syndrome</i> .....	223
<i>Gene therapy for lung injury</i> .....	223
<i>Gene therapy for bronchopulmonary dysplasia</i> .....	223
Concluding remarks about gene therapy of lungs .....	224
Companies involved in pulmonary gene therapy .....	224
<b>Skin and soft tissue disorders</b> .....	<b>225</b>
Gene transfer to the skin .....	225
<i>Electroporation for transdermal delivery of plasmid DNA</i> .....	225
<i>Electroporation for transdermal delivery of DNA vaccines</i> .....	225
<i>Liposomes for transdermal gene delivery</i> .....	226
<i>Ultrasound and topical gene therapy</i> .....	226
Gene therapy in skin disorders .....	226
<i>Gene therapy of hair loss</i> .....	226
<i>Gene therapy for epidermolysis bullosa</i> .....	227
<i>Gene therapy for xeroderma pigmentosum</i> .....	228
<i>Gene therapy for lamellar ichthyosis</i> .....	229
Gene transfer techniques for wound healing .....	229
<b>Urogenital disorders</b> .....	<b>230</b>
Gene therapy for urinary tract dysfunction .....	230
Gene therapy for erectile dysfunction .....	230
<i>NOS gene transfer for erectile dysfunction</i> .....	230
<i>Clinical trial of hMaxi-K Gene transfer in erectile dysfunction</i> .....	230
<i>Gene therapy for erectile dysfunction due to nerve injury</i> .....	231
<i>Concluding remarks on gene therapy for erectile dysfunction</i> .....	231
<b>Gene therapy of miscellaneous disorders</b> .....	<b>231</b>
Primary Sjögren's syndrome.....	231
<b>Veterinary gene therapy</b> .....	<b>232</b>
Gene therapy for mucopolysaccharidosis VII in dogs .....	232
Gene therapy to increase disease resistance.....	232
Gene therapy for infections .....	233
Gene therapy for chronic anemia .....	233
Gene therapy for endocrine disorders .....	233
Gene therapy for arthritis.....	234
Cancer gene therapy .....	234
<i>Brain tumors in cats and dogs</i> .....	234
<i>Breast cancer in dogs</i> .....	235
<i>Canine hemangiosarcoma</i> .....	235
<i>Canine melanoma</i> .....	236
<i>Canine soft tissue sarcoma</i> .....	236
Melanoma in horses .....	236
<b>4. Gene Therapy of Genetic Disorders</b> .....	<b>237</b>
<b>Introduction</b> .....	<b>237</b>
<b>Primary immunodeficiency disorders</b> .....	<b>238</b>
Severe combined immune deficiency .....	239
<i>Gene therapy for SCID</i> .....	239
<i>Lentiviral gene therapy combined with low-dose busulfan for SCID-X1</i> .....	242
Chronic granulomatous disease .....	242
Wiskott-Aldrich syndrome .....	243
Purine nucleoside phosphorylase deficiency .....	244
Major histocompatibility class II deficiency .....	244
Future prospects of gene therapy of inherited immunodeficiencies .....	244
<b>Metabolic disorders</b> .....	<b>245</b>
Alpha1-antitrypsin deficiency .....	245
<i>AAV mediated gene therapy for α1-antitrypsin deficiency</i> .....	246
<i>iPSCs for targeted gene correction of α1-antitrypsin deficiency</i> .....	247
<i>Gene editing for AAT deficiency</i> .....	248
Adrenoleukodystrophy .....	249
Canavan disease.....	250
Lesch-Nyhan syndrome .....	250
Lipoprotein lipase deficiency .....	251
<i>Alipogene tiparvovec</i> .....	251
Ornithine transcarbamylase deficiency .....	251
<i>Gene therapy for OTCD</i> .....	252
<i>AAV-mediated gene editing for OTCD using CRISPR-Cas9</i> .....	252

Phenylketonuria .....	253
Porphyrias .....	253
Tetrahydrobiopterin deficiency .....	254
<b>Lysosomal storage disorders .....</b>	<b>254</b>
Batten disease .....	255
Fabry's disease .....	256
Farber's disease .....	257
Gaucher disease .....	257
<i>Animals models of Gaucher's disease.</i> .....	257
<i>Gene therapy of Gaucher's disease.</i> .....	258
Glycogen storage disorders .....	258
<i>Glycogen storage disease type I</i> .....	258
<i>Gene therapy of GSD1a</i> .....	259
Hunter syndrome .....	259
Krabbe's disease .....	260
Metachromatic leukodystrophy .....	260
Mucopolysaccharidosis type 1 (Hurler syndrome) .....	261
Niemann-Pick type A disease .....	262
Pompe disease .....	262
Sanfilippo A syndrome .....	263
Sly syndrome .....	264
Tay-Sachs disease/Sandhoff disease .....	264
Future of gene therapy of lysosomal storage disorders .....	265
<b>Trinucleotide repeat disorders .....</b>	<b>265</b>
<b>Muscular dystrophies .....</b>	<b>265</b>
Duchenne muscular dystrophy (DMD) .....	265
<i>Animal models for gene therapy of DMD</i> .....	266
<i>Antisense approaches to DMD</i> .....	266
<i>CRISPR/Cas9 gene editing for DMD</i> .....	267
<i>Exon-skipping for DMD</i> .....	267
<i>Galgt2 gene therapy</i> .....	268
<i>Liposome-mediated gene transfer</i> .....	269
<i>Microdystrophin gene therapy</i> .....	269
<i>Myoblast-based gene transfer in DMD</i> .....	269
<i>Plasmid-mediated gene therapy</i> .....	270
<i>Purinoreceptor P2RX7 ablation</i> .....	270
<i>Post-transcriptional modulation of gene expression in DMD</i> .....	270
<i>Repair/Editing of dystrophin gene</i> .....	270
<i>Routes of administration of gene therapy in DMD</i> .....	273
<i>Types of dystrophin constructs</i> .....	274
<i>Viral vectors for DMD</i> .....	274
<i>Conclusions and future of gene therapy of DMD</i> .....	275
Limb-girdle muscular dystrophy .....	276
Myotonic dystrophy .....	276
Spinal muscular atrophy .....	277
<i>Gene therapy strategies for SMA</i> .....	277
<i>Zolgensma® gene therapy for SMA</i> .....	277
<i>Antisense approaches for SMA</i> .....	278
<i>Nusinersen</i> .....	278
<i>Nusinersen vs Zolgensma® gene therapy for SMA</i> .....	279
X-linked myotubular myopathy (XLMTM) .....	279
<b>Hereditary neuropathies .....</b>	<b>279</b>
Charcot-Marie-Tooth disease .....	279
Hereditary axonal neuropathies of the peripheral nerves .....	280
<b>Gene editing for hereditary transthyretin amyloidosis .....</b>	<b>280</b>
Current therapies for ATTR amyloidosis .....	280
CRISPR-Cas9 gene editing for ATTR amyloidosis .....	281
<b>Gene therapy of mitochondrial disorders .....</b>	<b>281</b>
Techniques for mitochondrial replacement therapy .....	282
Status of mitochondrial replacement and transfer therapies .....	283
Barth syndrome .....	284
<b>Companies involved in gene therapy of genetic disorders .....</b>	<b>284</b>
<b>5. Gene Therapy of Cancer .....</b>	<b>285</b>
<b>Strategies for cancer gene therapy .....</b>	<b>285</b>
<b>Direct gene delivery to the tumor .....</b>	<b>286</b>
Injection into tumor .....	286
<i>Direct injection of adenoviral vectors</i> .....	286
<i>Direct injection of a plasmid DNA-liposome complex</i> .....	287
<i>A polymer approach to local gene therapy for cancer</i> .....	287
Electroporation for cancer gene therapy .....	287

Control of gene expression in tumor by local heat .....	288
Radiation-guided gene therapy of cancer .....	288
Radioprotective gene therapy of cancer .....	288
Nanoparticles to facilitate combination of hyperthermia and gene therapy.....	289
<b>Immunogene therapy of cancer .....</b>	<b>289</b>
<b>Cell-based cancer gene therapy .....</b>	<b>290</b>
Adoptive cell therapy.....	290
CAR-T cell therapy for cancer .....	291
<i>CAR-T cells for tumor targeting.....</i>	291
<i>CAR-T cells targeting both CD19 and CD22 .....</i>	291
<i>Remote control of CAR-T cells for cancer immunotherapy .....</i>	291
<i>CAR-T cell therapy for leukemia.....</i>	292
<i>CAR-T cell therapy for multiple myeloma .....</i>	293
<i>CAR-T cell therapy for lymphoma.....</i>	293
<i>CAR-T cell therapy for solid tumors .....</i>	294
Cytokine gene therapy.....	295
Genetic modification of human hematopoietic stem cells .....	298
<b>Cancer vaccines .....</b>	<b>298</b>
Genetically modified cancer cell vaccines .....	298
<i>GVAX cancer vaccines .....</i>	299
<i>Genetically modified dendritic cells.....</i>	299
Nucleic acid-based cancer vaccines .....	300
<i>DNA cancer vaccines .....</i>	300
<i>RNA vaccines.....</i>	300
Viral vector-based cancer vaccines .....	300
<i>Intradermal delivery of cancer vaccines by Ad vectors .....</i>	301
Vaccines based on genetically engineered nonviral vectors.....	301
Future of cancer vaccines.....	301
Companies involved in nucleic acid-based cancer vaccines .....	302
<b>Monoclonal antibody gene transfer for cancer .....</b>	<b>303</b>
<b>Transfer and expression of intracellular adhesion-1 molecules .....</b>	<b>303</b>
<b>Other gene therapy techniques for immunotherapy of cancer .....</b>	<b>303</b>
Chemokines .....	303
CRISPR for immunogene therapy .....	304
Engineered viruses as anticancer immunotherapy vectors .....	305
Fas (Apo-1).....	305
Major Histocompatibility Complex (MHC) Class I .....	305
IGF (Insulin-Like Growth Factor).....	305
<b>Cancer immunotherapies targeting multiple mechanisms.....</b>	<b>306</b>
<b>Inhibition of immunosuppressive function in cancer .....</b>	<b>306</b>
<b>Delivery of toxic genes to tumor cells for eradication .....</b>	<b>306</b>
Gene-directed enzyme prodrug therapy .....	306
<b>Combination of gene therapy with radiotherapy .....</b>	<b>307</b>
<b>Correction of genetic defects in cancer cells .....</b>	<b>307</b>
<b>Targeted gene therapy for cancer .....</b>	<b>308</b>
Antiangiogenic therapy for cancer .....	308
Bacteria as novel anticancer gene vectors .....	309
Cancer-specific gene expression .....	309
Cancer-specific transcription.....	310
Delivery of retroviral particles hitchhiking on T cells .....	310
Electrogene and electrochemotherapy .....	310
Epidermal growth factor-mediated DNA delivery .....	310
Gene expression in hypoxic tumor cells .....	311
Genetically modified T cells for targeting tumors.....	311
Genetically engineered stem cells for targeting tumors .....	312
Hematopoietic stem cells for targeted cancer gene therapy .....	312
Immunolipoplex for delivery of p53 gene .....	313
Nanomagnets for targeted cell-based cancer gene therapy.....	313
Nanoparticles for targeted site-specific delivery of anticancer genes .....	314
Targeted cancer therapy using a dendrimer-based synthetic vector.....	314
Tumor-targeted gene therapy by receptor-mediated endocytosis .....	314
<b>Virus-mediated oncolysis.....</b>	<b>315</b>
Cytokine-induced killer cells for delivery of an oncolytic virus .....	315
Monitoring of viral-mediated oncolysis by PET .....	315
Oncolytic adenoviruses .....	316
Oncolytic HSV.....	317
Oncolytic measles viruses .....	317
Oncolytic vaccinia virus.....	318
Oncolytic vesicular stomatitis virus.....	318
Targeted cancer treatments based on oncolytic viruses.....	318
Concluding remarks on oncolytic gene therapy .....	319

Companies developing oncolytic viruses.....	319
<b>Apoptotic approach to improve cancer gene therapy .....</b>	<b>320</b>
<b>Tumor suppressor gene therapy .....</b>	<b>321</b>
P53 gene therapy.....	321
BRIT1 gene therapy .....	321
<b>Nitric oxide-based cancer gene therapy.....</b>	<b>321</b>
Anticancer effect of nitric oxide synthase .....	321
Gene therapy for radiosensitization of cancer .....	322
<b>Gene therapy of cancer of selected organs .....</b>	<b>322</b>
Gene therapy for bladder cancer .....	322
Gene therapy for glioblastoma .....	323
<i>Adenoviral vectors for treatment of brain tumors .....</i>	<i>324</i>
<i>Antiangiogenic gene therapy .....</i>	<i>324</i>
<i>Autophagy induced by conditionally replicating adenoviruses .....</i>	<i>325</i>
<i>Baculovirus vector for diphtheria toxin gene therapy .....</i>	<i>325</i>
<i>Cerepro® (sitimagene ceradenovec) .....</i>	<i>325</i>
<i>Gene therapy targeting hepatocyte growth factor.....</i>	<i>326</i>
<i>Genetically engineered MSCs for gene delivery to intracranial gliomas .....</i>	<i>326</i>
<i>Intravenous gene delivery with nanoparticles into brain tumors .....</i>	<i>326</i>
<i>Ligand-directed delivery of dsRNA molecules targeted to EGFR.....</i>	<i>327</i>
<i>Olig2 targeting to hinder growth of treatment-resistant glioblastomas .....</i>	<i>327</i>
<i>Oncolytic immunotherapy for pediatric high-grade gliomas .....</i>	<i>327</i>
<i>RNAi gene therapy of brain cancer .....</i>	<i>327</i>
<i>Targeting normal brain cells with an AAV vector encoding interferon-β .....</i>	<i>328</i>
<i>Viral oncolysis of glioblastoma multiforme.....</i>	<i>328</i>
Gene therapy for breast cancer.....	330
<i>Gene vaccine for breast cancer .....</i>	<i>330</i>
<i>Recombinant adenoviral ErbB-2/neu vaccine .....</i>	<i>331</i>
Gene Therapy for ovarian cancer .....	331
Gene therapy for malignant melanoma .....	332
<i>Immunogene therapy .....</i>	<i>333</i>
<i>Nonviral immunogene therapy for malignant melanoma.....</i>	<i>334</i>
<i>Oncogene targeted therapy.....</i>	<i>334</i>
<i>Targeted gene therapy for malignant melanoma .....</i>	<i>334</i>
Gene therapy of lung cancer.....	334
<i>Intravenous nanoparticle formulation for delivery of FUS1 gene .....</i>	<i>335</i>
<i>Aerosol gene delivery for lung cancer .....</i>	<i>335</i>
Gene therapy for cancer of prostate .....	335
<i>Experimental studies .....</i>	<i>335</i>
<i>Nanoparticle-based gene therapy for prostate cancer .....</i>	<i>336</i>
<i>Tumor suppressor gene therapy in prostate cancer .....</i>	<i>336</i>
<i>Vaccines for prostate cancer.....</i>	<i>336</i>
<i>Viral oncolysis for prostate cancer .....</i>	<i>337</i>
<i>Clinical trials of gene therapy for prostate cancer .....</i>	<i>337</i>
Gene therapy of head and neck cancer .....	337
<i>Adenoviral vector based P53 gene therapy .....</i>	<i>338</i>
<i>Gene therapy as adjunct to 5-FU for nasopharangeal carcinoma .....</i>	<i>338</i>
Gene therapy of pancreatic tumors .....	338
<i>Pancreatic neuroendocrine tumors .....</i>	<i>338</i>
<i>Pancreatic ductal adenocarcinoma.....</i>	<i>339</i>
<i>Editing of altered genes .....</i>	<i>339</i>
<i>Targeted gene therapy .....</i>	<i>340</i>
<i>Targeting in pancreatic adenocarcinoma with cell surface antigens.....</i>	<i>340</i>
<i>Targeted Expression of BikDD gene.....</i>	<i>340</i>
<i>Viral oncolysis in pancreatic cancer .....</i>	<i>341</i>
<i>Concluding remarks on gene therapy of pancreatic cancer .....</i>	<i>341</i>
Gene therapy of renal cancer.....	341
<i>Viral oncolytic therapy for renal cancer .....</i>	<i>341</i>
Gene therapy of hematological malignancies .....	341
<i>Genetically engineered T lymphocytes .....</i>	<i>341</i>
<b>Cancer gene therapy companies .....</b>	<b>342</b>

## **6. Gene Therapy of Neurological Disorders.....**

<b>Indications .....</b>	<b>345</b>
<b>Gene transfer techniques for the nervous system.....</b>	<b>346</b>
Methods of gene transfer to the nervous system.....	346
Ideal vector for gene therapy of neurological disorders .....	346
Promoters of gene transfer.....	346
Lentivirus-mediated gene transfer to the CNS .....	347
AAV vector mediated gene therapy for neurogenetic disorders.....	347
Gene transfer to the CNS using recombinant SV40-derived vectors .....	348

Routes of delivery of genes to the nervous system.....	348
<i>Direct injection into CNS</i> .....	348
<i>Introduction of the genes into cerebral circulation</i> .....	349
<i>Introduction of genes into cerebrospinal fluid</i> .....	349
<i>Intravenous administration of vectors</i> .....	350
Delivery of gene therapy to the peripheral nervous system.....	350
Cell-mediated gene therapy of neurological disorders .....	350
<i>Neuronal cells</i> .....	350
<i>Neural stem cells and progenitor cells</i> .....	350
<i>Astrocytes</i> .....	351
<i>Cerebral endothelial cells</i> .....	351
<i>Implantation of genetically modified encapsulated cells into the brain</i> .....	351
Gene transfer for neuromodulation.....	352
<b>Monitoring of CNS gene therapy .....</b>	<b>352</b>
<b>Gene therapy of neurodegenerative disorders .....</b>	<b>352</b>
Gene therapy for Parkinson disease.....	352
Rationale .....	353
<i>Techniques of gene therapy for PD</i> .....	354
<i>Augmenting CNS glucocerebrosidase activity</i> .....	357
<i>Delivery of neurotrophic factors by gene therapy</i> .....	357
<i>Delivery of parkin gene</i> .....	358
<i>Gene editing in PD</i> .....	358
<i>Introduction of functional genes into the brain of patients with PD</i> .....	358
<i>Nanoparticle-based gene therapy for PD</i> .....	359
<i>Mitochondrial gene therapy for PD</i> .....	359
<i>RNAi approach to PD</i> .....	359
<i>Viral vector-based ubiquitination to prevent spread of <math>\alpha</math>-synuclein</i> .....	360
<i>Prospects of gene therapy for PD</i> .....	360
<i>Concluding remarks about gene therapy of PD</i> .....	361
<i>Companies developing gene therapy for PD</i> .....	361
Gene therapy for Alzheimer disease .....	362
Rationale .....	362
<i>NGF gene therapy for AD</i> .....	362
<i>FGF2 gene transfer in AD</i> .....	363
Gene therapy for restoring brain cholesterol metabolism.....	363
<i>Neprilysin gene therapy</i> .....	364
<i>Viral gene transfer of APPsa for rescuing synaptic failure in AD</i> .....	364
<i>Gene vaccination</i> .....	365
<i>Combination of gene therapy with other treatments for AD</i> .....	365
Gene therapy of Huntington disease.....	365
<i>Encapsulated genetically engineered cellular implants</i> .....	365
<i>Viral vector mediated administration of neurotrophic factors</i> .....	366
<i>RNAi gene therapy</i> .....	366
Gene therapy of amyotrophic lateral sclerosis.....	366
Rationale .....	366
<i>Technique of gene therapy of ALS</i> .....	366
<i>Other approaches to gene therapy of ALS</i> .....	368
<b>Gene therapy of cerebrovascular diseases.....</b>	<b>368</b>
Preclinical research in gene therapy for cerebrovascular disease .....	368
Animal models of stroke relevant to gene therapy .....	369
<i>Transgenic mice as models for stroke</i> .....	369
<i>Animal models for gene therapy of arteriovenous malformations</i> .....	369
Gene transfer to cerebral blood vessels .....	369
Gene therapy for vasospasm following subarachnoid hemorrhage .....	370
<i>NOS gene therapy for cerebral vasospasm</i> .....	371
Gene therapy for stroke.....	372
<i>Gene therapy for stroke using neurotrophic factors</i> .....	373
<i>Gene therapy of strokes with a genetic component</i> .....	373
<i>Gene therapy for intracranial aneurysms</i> .....	373
<i>RNAi-based gene silencing for neuroprotection in cerebral ischemia</i> .....	374
Concluding remarks about gene therapy for stroke.....	374
<b>Gene therapy of injuries to the nervous system.....</b>	<b>374</b>
Traumatic brain injury .....	374
Spinal cord injury.....	375
Peripheral nerve injuries .....	376
<b>Gene therapy of epilepsy .....</b>	<b>376</b>
Gene therapy for control of seizures .....	376
Gene therapy for neuroprotection in epilepsy .....	377
Gene therapy for genetic forms of epilepsy .....	378
<b>Gene therapy for multiple sclerosis .....</b>	<b>378</b>
<b>Gene therapy for impairment of special senses .....</b>	<b>378</b>

Gene therapy for hearing loss.....	379
Gene therapy for olfactory impairment .....	379
<b>Gene therapy for relief of pain .....</b>	<b>380</b>
Rationale of gene therapy for pain .....	380
Vectors for gene therapy of pain .....	380
Methods of gene delivery for pain .....	381
<i>Endogenous analgesic production for cranial neuralgias .....</i>	<i>381</i>
<i>Gene delivery by intrathecal route .....</i>	<i>382</i>
<i>Gene transfer for delivery of analgesics to the spinal nerve roots .....</i>	<i>382</i>
<i>Gene therapy of peripheral neuropathic pain .....</i>	<i>383</i>
<i>Gene transfer by injections into the brain substance.....</i>	<i>384</i>
Targets for gene therapy of pain.....	384
<i>Zinc finger DNA-binding protein therapeutic for chronic pain .....</i>	<i>384</i>
<i>Gene therapy for producing enkephalin to block pain signals .....</i>	<i>385</i>
<i>Targeting nuclear factor-<math>\kappa</math>B .....</i>	<i>385</i>
<i>Gene therapy targeted to neuroimmune component of chronic pain .....</i>	<i>385</i>
Potential applications of gene therapy for management of pain.....	386
Concluding remarks on gene therapy for pain.....	386
<b>Gene therapy for psychiatric disorders .....</b>	<b>387</b>
Gene therapy for depression .....	387
Gene therapy for enhancing cognition after stress.....	388
Gene therapy against fear disorders .....	388
<b>Companies involved in gene therapy of neurological disorders.....</b>	<b>389</b>
<b>7. Gene Therapy of Cardiovascular Disorders .....</b>	<b>391</b>
<b>Introduction .....</b>	<b>391</b>
<b>Techniques of gene transfer to the cardiovascular system .....</b>	<b>391</b>
Direct plasmid injection into the myocardium .....	392
Catheter-based systems for vector delivery .....	392
Ultrasound microbubbles for cardiovascular gene delivery.....	393
Vectors for cardiovascular gene therapy .....	393
<i>AAV vectors for therapeutic delivery to the heart .....</i>	<i>393</i>
<i>Adenoviral vectors for cardiovascular diseases.....</i>	<i>394</i>
<i>Molecular cardiac surgery with recirculating delivery of AAV vectors .....</i>	<i>394</i>
<i>Plasmid DNA-based delivery in cardiovascular disorders.....</i>	<i>394</i>
Gene therapy for counteracting hypoxia in myocardial ischemia.....	394
Therapeutic angiogenesis vs vascular growth factor therapy.....	395
Gene painting for delivery of targeted gene therapy to the heart .....	395
Gene delivery to vascular endothelium .....	396
Targeted plasmid DNA delivery to the cardiovascular system with nanoparticles .....	396
Vascular stents for gene delivery .....	397
<b>Gene therapy for genetic cardiovascular disorders .....</b>	<b>397</b>
Catecholaminergic polymorphic ventricular tachycardia.....	397
Genetic disorders predisposing to atherosclerosis.....	398
Familial hypercholesterolemia.....	399
Apolipoprotein E deficiency.....	400
Hypertension .....	400
Genetic factors for myocardial infarction .....	401
<b>Acquired cardiovascular diseases .....</b>	<b>402</b>
Coronary artery disease with angina pectoris.....	402
<i>Ad5FGF-4.....</i>	<i>402</i>
Gene therapy for improving long-term CABG patency rates.....	403
Ischemic heart disease with myocardial infarction .....	403
<i>Gene therapy and angiogenesis in ischemic heart disease .....</i>	<i>403</i>
<i>Induction of angiogenesis in myocardium by HEXIM1 re-expression .....</i>	<i>404</i>
<i>Myocardial repair with IGF-1 therapy.....</i>	<i>405</i>
<i>Metalloproteinase-2 inhibitor gene therapy.....</i>	<i>405</i>
<i>miRNA gene therapy for ischemic heart disease .....</i>	<i>406</i>
Congestive heart failure.....	406
<i>Rationale of gene therapy in CHF .....</i>	<i>406</i>
<i>AAV-mediated gene transfer for CHF .....</i>	<i>406</i>
<i>AngioCell gene therapy for CHF .....</i>	<i>407</i>
<i><math>\beta</math>-ARKct gene therapy.....</i>	<i>408</i>
<i>Elevating cardiac dATP by gene therapy to improve cardiac function.....</i>	<i>408</i>
<i>Elevating cardiac adenyl cyclase type 6 to improve cardiac function.....</i>	<i>409</i>
<i>Intracoronary adenovirus-mediated gene therapy for CHF.....</i>	<i>409</i>
<i>nNOS gene transfer in CHF.....</i>	<i>410</i>
Cardiomyopathies .....	410
Cardiac arrhythmias .....	410
<i>Gene transfer approaches for biological pacemakers.....</i>	<i>411</i>
<i>Genetically engineered biological pacemakers.....</i>	<i>412</i>

<i>Gene therapy for ventricular arrhythmia .....</i>	412
Gene therapy and heart transplantation.....	412
Hyperlipidemia and hypercholesterolemia .....	413
<i>Antisense approach to hypertriglyceridemia.....</i>	413
<i>Cholesterol reduction by genetic engineering of PCSK9 gene .....</i>	413
<i>Inactivating variants in ANGPTL4 for lowering circulating triglycerides .....</i>	414
<b>Peripheral arterial disease.....</b>	<b>414</b>
Incidence and clinical features .....	414
Current management .....	414
Gene therapy for peripheral arterial disease .....	415
<i>Angiogenesis by gene therapy .....</i>	415
<i>HIF-1<math>\alpha</math> gene therapy for peripheral arterial disease .....</i>	415
<i>HGF gene therapy for peripheral arterial disease.....</i>	416
Prevention of restenosis after angioplasty .....	416
<i>Antisense approaches.....</i>	416
<i>Gene therapy to prevent restenosis after angioplasty .....</i>	417
<i>hTIMP-1 gene therapy to prevent intimal hyperplasia .....</i>	418
<i>miRNA-based gene therapy for restenosis .....</i>	418
<i>NOS gene therapy for restenosis.....</i>	419
<i>Techniques of gene therapy for restenosis.....</i>	419
Maintaining vascular patency after surgery .....	420
<b>Companies involved in gene therapy of cardiovascular diseases .....</b>	<b>420</b>
Future of gene therapy of cardiovascular disorders.....	421
<b>8. Gene therapy of viral infections .....</b>	<b>423</b>
<b>Introduction .....</b>	<b>423</b>
<b>Acquired Immunodeficiency Syndrome (AIDS).....</b>	<b>423</b>
Current management of AIDS.....	423
Gene therapy strategies in HIV/AIDS.....	424
Cell/gene therapies for HIV/AIDS.....	425
<i>Anti-HIV ribozyme delivered in hematopoietic progenitor cells .....</i>	425
<i>Autocrine interferon (INF)-<math>\beta</math> production by somatic cell gene therapy .....</i>	425
<i>Gene editing for HIV-1.....</i>	425
<i>Transplantation of genetically modified T cells .....</i>	426
<i>Transplantation of genetically modified hematopoietic cells .....</i>	427
Inhibition of HIV-1 replication by lentiviral vectors .....	428
<i>VRX496-T .....</i>	428
Insertion of protective genes into target cells .....	429
HIV/AIDS vaccines.....	429
Intracellular immunization.....	430
Engineered cellular proteins such as soluble CD4s .....	430
Intracellular antibodies .....	431
Anti-rev single chain antibody fragment .....	431
Use of genes to chemosensitize HIV-1 infected cells .....	431
Antisense approaches to AIDS .....	431
<i>RNA decoys.....</i>	431
<i>Antisense oligodeoxynucleotides .....</i>	432
<i>RNA decoys.....</i>	432
<i>Ribozymes .....</i>	432
RNAi applications in HIV/AIDS .....	433
<i>siRNA-directed inhibition of HIV-1 infection .....</i>	433
<i>Role of the nef gene during HIV-1 infection and RNAi .....</i>	434
<i>Bispecific siRNA constructs .....</i>	434
<i>Targeting CXCR4 with siRNAs .....</i>	434
<i>Targeting CCR5 with siRNAs .....</i>	434
Companies involved in developing gene therapy for HIV/AIDS .....	435
Conclusions regarding gene therapy of HIV/AIDS.....	436
<b>Genetic vaccines for other viral infections .....</b>	<b>436</b>
Cytomegalic virus infections .....	436
Viral hepatitis .....	437
<i>Vaccine for hepatitis B .....</i>	437
<i>Vaccine for hepatitis C .....</i>	437
<i>Gene therapy for hepatitis C .....</i>	438
Vaccine for herpes simplex virus .....	438
DNA vaccine against rabies .....	438
DNA vaccine for Ebola .....	439
Vaccines for avian influenza .....	439
<i>Future prospects of DNA vaccines for avian influenza .....</i>	440
<i>Human trial of a DNA vaccine for avian influenza .....</i>	441
Companies developing genetic vaccines for infections other than AIDS .....	441

<b>9. Research, Development and Future of Gene Therapy.....</b>	<b>443</b>
<b>Basic research in gene therapy.....</b>	<b>443</b>
<b>R &amp; D in gene therapy.....</b>	<b>443</b>
Animal models of human diseases for gene therapy research .....	444
<i>Lentiviral transgenesis.....</i>	444
<b>Financing research and development.....</b>	<b>444</b>
Role of the NIH in gene therapy research .....	444
National Gene Vector Laboratories .....	444
Funding of gene therapy research in Europe.....	445
<i>Gene therapy funding in Horizon 2020 of European Commission .....</i>	445
Financing by the industry.....	446
<b>Clinical trials in gene therapy .....</b>	<b>446</b>
Clinical trials worldwide .....	446
<i>Clinical trials in cancer gene therapy .....</i>	447
<i>Trials of gene therapy for neurological disorders.....</i>	447
<i>Clinical trials for genetic disorders.....</i>	447
<i>Clinical trials in cardiovascular gene therapy .....</i>	447
<i>Clinical trials for infectious diseases.....</i>	447
<i>Gene therapy for other disorders .....</i>	447
Clinical trials in the US.....	448
Vectors used in gene therapy clinical trials .....	448
<i>Vector analytics for clinical trials using rAAV vectors.....</i>	449
<b>Gene therapy in China.....</b>	<b>449</b>
<b>Future of gene therapy .....</b>	<b>450</b>
How to improve gene therapy.....	450
International Gene Therapy Consortium.....	451
Future opportunities and challenges for gene therapy .....	452
Promising areas of application of gene therapy .....	453
<i>Neurological disorders .....</i>	453
<i>Gene therapy of cardiovascular disorders.....</i>	454
<i>Cancer gene therapy .....</i>	455
Personalized gene therapy .....	456
<b>10. Regulatory, Safety, Ethical Patent Issues of Gene Therapy .....</b>	<b>457</b>
<b>Regulation of gene therapy in the United States.....</b>	<b>457</b>
US Federal guidelines for research involving recombinant DNA molecules .....	457
Regulation of gene therapy in US .....	457
Implantation of genetically manipulated cells.....	457
<i>Modification of oocytes for use in IVF.....</i>	458
Clinical trials in gene therapy .....	458
Gene therapy INDs placed on hold by the FDA.....	459
FDA policy for advancing development of gene therapy .....	459
<i>Future for approval of gene therapy in the US .....</i>	461
Do-it-yourself gene therapy .....	463
<b>Regulation of gene therapy in Europe.....</b>	<b>463</b>
European Union .....	463
Regulation of gene therapy in Germany .....	464
<i>Preclinical research .....</i>	464
<i>Clinical Trials .....</i>	465
Marketing authorization .....	466
Regulation of gene therapy in the United Kingdom .....	466
Regulation of gene therapy in France.....	467
Regulation of gene therapy in Italy .....	467
Regulation of gene therapy in the Netherlands.....	467
Gene therapy regulation in Switzerland.....	469
<b>Regulation of gene therapy in Australia.....</b>	<b>469</b>
<b>Regulation of gene therapy in Japan.....</b>	<b>470</b>
<b>Regulation of gene therapy in China .....</b>	<b>470</b>
<b>Safety issues of gene transfer .....</b>	<b>470</b>
Adverse effects of retroviral vectors .....	471
<i>Insertional mutagenesis.....</i>	471
Adverse effects of HSV vectors .....	471
<i>Neurotoxicity of HSV vectors .....</i>	471
<i>Hepatotoxicity of HSV-tk/ganciclovir approach.....</i>	472
Adverse effects of adenoviral vectors.....	472
<i>Inflammatory effects of adenoviruses in lungs .....</i>	472
<i>Inflammatory effects involving the liver .....</i>	472
<i>Induction of immune response by adenoviral vectors.....</i>	473
<i>Impairment of adrenocortical steroidogenesis.....</i>	473
<i>Adverse effects of AAV vectors .....</i>	473
Toxicity associated with cationic lipid-mediated gene transfer.....	474

Toxicity of lipopolysaccharides .....	474
Potential side effects of RNAi gene therapy.....	474
Role of molecular diagnostics in safety of gene therapy .....	475
Quality control of vectors .....	475
<i>Testing for retroviruses.....</i>	475
<i>Adenoviral vectors .....</i>	476
<i>Replication competent viruses .....</i>	476
Genetic characteristics of viral vectors .....	476
GMP-compliant viral vectors for human gene therapy .....	477
Concluding remarks about safety of viral vectors .....	477
<b>Ethical aspects of gene therapy .....</b>	<b>477</b>
The lay consumer's view of somatic gene therapy ethics .....	478
Ethical aspects of clinical trials.....	478
Regulatory and ethical issues for in utero gene therapy .....	478
Ethical aspects of germline gene therapy .....	479
Ethical aspects of mitochondrial replacement therapy .....	479
Ethical aspects of gene editing.....	480
<i>Ethical aspects of clinical trials of germline editing .....</i>	480
<i>Gene editing in the UK.....</i>	481
<i>Gene editing in the US.....</i>	482
<i>Guidelines for gene editing by Alliance for Regenerative Medicine .....</i>	483
<i>UNESCO's view on gene editing in humans.....</i>	484
<i>Concluding remarks on the ethical aspects of genome editing.....</i>	485
Germline gene therapy for genetic enhancement .....	485
Athletic enhancement by genetic engineering .....	485
<i>Gene doping in sports.....</i>	486
<i>Gene transfer methods used for enhancing physical performance.....</i>	486
<i>Adverse effect of genetic engineering .....</i>	488
<i>Problems in detecting genetic manipulations in athletes.....</i>	488
<i>Ethical dilemma .....</i>	488
<b>Gene therapy patents .....</b>	<b>489</b>
CRISP/Cas9 patents .....	489

## Tables

Table 1-1: Landmarks in development of gene therapy.....	23
Table 2-1: Classification of methods of gene therapy.....	35
Table 2-2: A comparison of various physical methods of gene transfer .....	37
Table 2-3: Experimental applications of gene transfer by electroporation .....	39
Table 2-4: An overview of characteristics of commonly used viral vectors .....	47
Table 2-5: Companies using viral vectors.....	60
Table 2-6: Companies using nonviral vectors .....	79
Table 2-7: Target organs for nonviral gene therapy methods. ....	81
Table 2-8: CRISPR vs other gene editing tools.....	88
Table 2-9: Companies developing CRISPR technology .....	100
Table 2-10: Companies developing CAR-T cell therapy .....	114
Table 2-11: Potential routes for administration of DNA .....	119
Table 2-12: Companies with gene delivery devices/ technologies .....	121
Table 2-13: Strategies for targeted gene therapy .....	122
Table 2-14: Animal experimental studies of in vivo gene delivery with polymer systems .....	123
Table 2-15: Approaches to controlling gene expression in gene therapy .....	124
Table 2-16: Companies with regulated/targeted gene therapy and special techniques .....	126
Table 2-17: Potential applications of human germline genome modification .....	128
Table 2-18: Applications of molecular diagnostics in gene therapy .....	141
Table 2-19: Advantages of gene therapy compared with protein therapy .....	144
Table 3-1: Experimental approaches to gene therapy of rheumatoid arthritis .....	149
Table 3-2: Gene therapy strategies for osteoarthritis.....	151
Table 3-3: Cell and gene therapy approaches for type 1 diabetes mellitus.....	158
Table 3-4: Indications for gastrointestinal gene therapy .....	164
Table 3-5: Hematological disorders that can be potentially treated by gene therapy.....	166
Table 3-6: Clinical trials of gene therapy for hemophilia A and B.....	168
Table 3-7: Companies involved in gene therapy of hematological disorders.....	179
Table 3-8: Techniques of gene transfer to the kidneys.....	185
Table 3-9: Gene therapy in animal experimental models of renal disease.....	187
Table 3-10: Applications of gene therapy in ophthalmological disorders .....	196
Table 3-11: Companies developing gene therapy for eye disorders .....	213
Table 3-12: Strategies for gene delivery to the lungs .....	217
Table 3-13: Companies developing gene therapy for pulmonary disorders .....	224
Table 4-1: Genetic disorders that are being investigated for gene therapy .....	237

Table 4-2: X-linked immunodeficiency disorders .....	239
Table 4-3: Examples of inherited metabolic disorders amenable to gene therapy .....	245
Table 4-4: Gene therapy approaches to Duchenne muscular dystrophy .....	266
Table 4-5: Companies involved in gene therapy of genetic/metabolic disorders .....	284
Table 5-1: Strategies for cancer gene therapy .....	285
Table 5-2: Cell-based gene therapy for cancer .....	290
Table 5-3: Companies with nucleic acids/genetically modified cell cancer vaccines .....	302
Table 5-4: Enzyme/prodrug combinations employed in suicide gene therapy .....	307
Table 5-5: Mutation compensation strategies used clinically .....	307
Table 5-6: Companies developing oncolytic viruses .....	319
Table 5-7: Strategies for gene therapy of malignant brain tumors.....	323
Table 5-8: Clinical trials of oncolytic virotherapy against glioblastoma multiforme.....	329
Table 5-9: Clinical trials of gene therapy in ovarian cancer.....	331
Table 5-10: Gene therapy for malignant melanoma.....	332
Table 5-11: Clinical trials of gene therapy for prostate cancer .....	337
Table 5-12: Companies involved in cancer gene therapy.....	342
Table 6-1: Example of potential indications for gene therapy of neurologic disorder .....	345
Table 6-2: Methods of gene transfer as applied to neurologic disorders .....	346
Table 6-3: Gene therapy techniques applicable to Parkinson disease .....	353
Table 6-4: Companies developing gene therapy for Parkinson's disease.....	361
Table 6-5: Gene transfer in animal models of carotid artery restenosis.....	370
Table 6-6: Gene therapy strategies for vasospasm.....	371
Table 6-7: Neuroprotective gene therapy in animal stroke models .....	372
Table 6-8: Experimental gene therapy approaches for relief of pain .....	381
Table 6-9: Companies involved in gene therapy of neurological disorders .....	389
Table 7-1: Cardiovascular disorders that are potential indications for gene therapy .....	391
Table 7-2: Catheter-based systems for vector delivery to the cardiovascular system .....	392
Table 7-3: Companies involved in gene therapy of cardiovascular diseases .....	420
Table 8-1: Strategies for gene therapy of AIDS .....	424
Table 8-2: Companies involved in developing gene therapy for HIV/AIDS .....	435
Table 8-3: Companies developing genetic vaccines for infections other than AIDS .....	441
Table 9-1: Geographical distribution of gene therapy clinical trials .....	446
Table 9-2: Opportunities and challenges for gene therapy and resources needed .....	453
Table 9-3: Potential applications of gene therapy in disorders of the nervous system .....	454
Table 10-1: Genes that may be used for performance enhancement .....	486

## Figures

Figure 1-1: Relation of gene therapy to other biotechnologies .....	26
Figure 1-2: Relationship of DNA, RNA and protein in the cell .....	30
Figure 2-1: Ex vivo and in vivo techniques of gene therapy.....	36
Figure 2-2: Structure of the Helios gene gun.....	42
Figure 2-3: Categories of rAAV-based gene therapy strategies .....	50
Figure 2-4: Gene editing by zinc finger nucleases .....	83
Figure 2-5: Genome engineering by transcription-activator-like-effector-nucleases.....	84
Figure 2-6: Mechanism of action of GeneRide technology.....	85
Figure 2-7: A scheme of CRISPR/Cas9 gene editing .....	86
Figure 2-8: Use of CRISPR-Cas9 for hereditary hearing loss .....	98
Figure 2-9: Chimeric antigen receptor (CAR)-T cells attacking tumor cells.....	113
Figure 2-10: Schematic of suppression of gene expression by RNAi.....	138
Figure 3-1: Retina and routes of administration of gene therapy for retinal disorders .....	202
Figure 4-1: Targeted gene correction of α1-antitrypsin deficiency by iPSCs .....	248
Figure 4-2: Application of CRISPR-Cas9 in a mouse model of DMD .....	272
Figure 4-3: Techniques for mitochondrial nuclear transfer.....	282
Figure 5-1: Gene therapy approaches for pancreatic cancer .....	339
Figure 6-1: Effect of tyrosine hydroxylase gene delivery on dopamine levels .....	354
Figure 6-2: Role of cell and gene therapy in stroke according to pathology and stage.....	374
Figure 9-1: Product development cycle in gene therapy .....	443
Figure 9-2: Proportions of therapeutic areas in clinical trials of gene therapy in the US .....	448
Figure 9-3: Proportions of various vectors used in gene therapy trials .....	448