

TABLE OF CONTENTS

0. Executive Summary	19
1. Introduction	21
Definitions	21
Historical evolution of gene therapy	21
Relation of gene therapy to other biotechnologies.....	23
Molecular biological basics for gene therapy	23
Genome.....	23
DNA	24
RNA	24
<i>Alternative RNA splicing.....</i>	<i>25</i>
Genes.....	26
Gene regulation	26
Gene expression	28
Chromosomes	28
Telomeres.....	29
Mitochondrial DNA	29
Proteins	30
2. Gene Therapy Technologies	31
Classification of gene therapy techniques.....	31
Ex vivo and in vivo gene therapy	32
Ex vivo gene therapy.....	32
In vivo gene therapy	33
Physical methods of gene transfer.....	33
Electroporation	33
Applications of electroporation.....	34
<i>Clinical applications of electroporation</i>	<i>35</i>
<i>Advantages of electroporation</i>	<i>35</i>
<i>Limitations of electroporation</i>	<i>36</i>
Hydrodynamic	36
Microinjection	36
Particle bombardment	37
Ultrasound-mediated transfection	39
Molecular vibration.....	40
Application of pulsed magnetic field and superparamagnetic nanoparticles	40
Gene transfection using laser irradiation	40
Photochemical transfection.....	41
Chemical methods of gene transfer	41
Gene repair and replacement.....	41
Gene repair by single-stranded oligonucleotides.....	42
History and current status of chimeraplasty	42
mRNA gene therapy	42
Spliceosome mediated RNA trans-splicing	43
Vectors for gene therapy	44
Basic considerations	44
Use of genes as pharmaceuticals	44
The ideal vector for gene therapy.....	44
Viral vectors.....	45
<i>Adenovirus vectors.....</i>	<i>46</i>
<i>Adeno-associated virus vectors.....</i>	<i>48</i>
<i>Alphavirus vectors.....</i>	<i>50</i>
<i>Baculovirus vectors</i>	<i>50</i>
<i>Foamy virus vectors</i>	<i>51</i>
<i>Herpes simplex virus vectors.....</i>	<i>51</i>
<i>Lentiviral vectors</i>	<i>53</i>
<i>Multicistronic retroviral vectors</i>	<i>54</i>
<i>Retroviral vectors.....</i>	<i>55</i>
<i>Oncogenic potential of retroviral vectors.....</i>	<i>56</i>
<i>Future prospects of viral vectors</i>	<i>57</i>
<i>Companies using viral vectors</i>	<i>57</i>
Nonviral vectors for gene therapy	59
<i>Effects of shape of DNA molecules on delivery with nonviral vectors.....</i>	<i>59</i>
<i>Anionic lipid-DNA complexes</i>	<i>59</i>
<i>Cationic lipid-DNA complexes</i>	<i>60</i>
<i>Liposomes for gene therapy</i>	<i>60</i>
<i>Liposome-nucleic acid complexes.....</i>	<i>62</i>

<i>Liposome-HVJ complex</i>	62
<i>Mobile DNA vectors</i>	63
<i>Polycation-DNA complexes (polyplexes)</i>	63
<i>Plasmid DNA vector for treatment of chronic inflammatory disease</i>	64
<i>Polymer molecules</i>	64
<i>Synthetic peptide complexes</i>	64
<i>Future prospects of nonviral vs viral vectors</i>	65
Nanobiotechnology for gene transfer	65
<i>Nanoparticles as nonviral vectors for gene therapy</i>	65
<i>Dendrimers</i>	66
<i>Cochleates</i>	66
<i>Calcium phosphate nanoparticles as non-viral vectors</i>	67
<i>Lipid nanoparticles for nucleic acid delivery</i>	67
<i>Silica nanoparticles as a nonviral vector for gene delivery</i>	68
<i>Gelatin nanoparticles for gene delivery</i>	68
<i>Nonionic polymeric micelles for oral gene delivery</i>	68
<i>Biological nanoparticle technology</i>	69
<i>Nanoparticles with virus-like function as gene therapy vectors</i>	69
Receptor-mediated endocytosis	69
Artificial viral vectors	70
Directed evolution of AAV to create efficient gene delivery vectors	71
Bacterial ghosts as DNA delivery systems	71
Bacteria plus nanoparticles for gene delivery into cells.....	72
Chromosome-based vectors for gene therapy	73
Companies using nonviral vectors	74
Concluding remarks about vectors	75
Cell-mediated gene therapy	76
Fibroblasts	76
Skeletal muscle cells	77
Vascular smooth muscle cells	78
Keratinocytes	78
Hepatocytes	78
Lymphocytes	78
<i>Regulating protein delivery by genetically encoded lymphocytes</i>	79
Implantation of microencapsulated genetically modified cells	79
Stem cell gene therapy	80
<i>Therapeutic applications for hematopoietic stem cell gene transfer</i>	80
<i>Improving delivery of genes to stem cells</i>	80
<i>Lentiviral vectors for gene transfer to marrow stem cells</i>	81
<i>Use of mesenchymal stem cells for gene therapy</i>	81
<i>In utero gene therapy using stem cells</i>	81
<i>Gene delivery to stem cells by artificial chromosome expression</i>	81
<i>Linker based sperm-mediated gene transfer technology</i>	82
<i>Combination of gene therapy with therapeutic cloning</i>	82
<i>Expansion of transduced HSCs in vivo</i>	82
<i>The future of hematopoietic stem cell gene therapy</i>	82
Routes of administration for gene therapy	83
Direct injection of naked DNA	83
Intramuscular injection	84
Intravenous DNA injection	84
Intraarterial delivery	84
Companies with gene delivery devices/ technologies	84
Targeted gene therapy	85
Targeted integration.....	86
Bacteriophage integrase system for site-specific gene delivery	86
Controlled-release delivery of DNA	86
Controlled gene therapy	87
Controlled delivery of genetic material	87
Controlled induction of gene expression	87
Drug-inducible systems for control of gene expression.....	88
Timed activation of gene therapy by a circuit based on signaling network	89
Small molecules for post-transcriptional regulation of gene expression	89
Engineered zinc finger DNA binding proteins for gene correction	89
Light Activated Gene Therapy	90
Spatial control of gene expression via local hyperthermia.....	90
Companies with regulated /targeted gene therapy	90
Gene marking	91
Germline gene therapy	92
Potential applications of human germline genome modification	92
Pros and cons of human germline genome modification	92
Role of gene transfer in antibody therapy	94

Genetically engineered vaccines	94
DNA vaccines	94
<i>DNA inoculation technology</i>	95
<i>Methods for enhancing the potency of DNA vaccines</i>	95
<i>Advantages of DNA vaccines</i>	96
Vaccine vectors	96
Challenges and limitations of genetically engineered vaccines	97
Vaccines based on reverse genetics	97
Technologies for gene suppression	98
Antisense oligonucleotides	98
Transcription factor decoys	99
Aptamers	99
Ribozymes	99
Peptide nucleic acid	100
<i>Intracellular delivery of PNAs</i>	100
Locked nucleic acid	100
<i>Zorro-LNA</i>	101
Gene silencing	101
Post-transcriptional gene silencing	101
Definitions and terminology of RNAi	102
RNAi mechanisms	102
Inhibition of gene expression by antigenic RNA	103
RNAi gene therapy	103
Application of molecular diagnostic methods in gene therapy	104
Use of PCR to study biodistribution of gene therapy vector	104
PCR for verification of the transcription of DNA	104
In situ PCR for direct quantification of gene transfer into cells	104
Detection of retroviruses by reverse transcriptase (RT)-PCR	105
Confirmation of viral vector integration	105
Monitoring of gene expression	105
<i>Monitoring of gene expression by green fluorescent protein</i>	105
<i>Monitoring in vivo gene expression by molecular imaging</i>	106
<i>Advantages of gene therapy compared with protein therapy</i>	106
3. Clinical Applications of Gene Therapy	107
Introduction	107
Bone and joint disorders	107
Bone fractures	107
Gene therapy for intervertebral disc degeneration	108
Spinal fusion	108
Osteogenesis imperfecta	109
Rheumatoid arthritis	109
<i>Local or systemic treatment</i>	110
<i>In vivo or ex vivo gene therapy of RA</i>	110
<i>Clinical trials</i>	111
Gene therapy for osteoarthritis	112
Sports injuries	113
<i>Repair of articular cartilage defects</i>	113
Regeneration and replacement of bone by gene therapy	114
Bacterial infections	115
Antisense approach to bacterial infections	115
Dentistry	115
Tissue engineering in dental implant defects	115
Endocrine disorders	116
Introduction	116
Diabetes mellitus	116
Methods of gene therapy of diabetes mellitus	116
<i>Viral vector-mediated gene transfer in diabetes</i>	117
<i>Gene delivery with ultrasonic microbubble destruction technology</i>	118
<i>Genetically engineered cells for diabetes mellitus</i>	118
<i>Genetically altered liver cells</i>	118
<i>Genetically modified stem cells</i>	119
<i>Genetically engineered dendritic cells</i>	119
<i>Insertion of gene encoding for IL-4</i>	119
<i>Concluding remarks about cell and gene therapy of diabetes</i>	119
Gene therapy of growth-hormone deficiency	120
Gene therapy of obesity	121
<i>Ad viral vector-mediated transfer of leptin gene</i>	121
<i>AAV vector-mediated delivery of GDNF for obesity</i>	122
Gastrointestinal disorders	122
Introduction	122

Methods of gene transfer to the gastrointestinal tract	123
<i>Direct delivery of genes</i>	123
<i>Naked plasmid DNA into the submucosa</i>	123
<i>Viral vectors</i>	123
<i>Receptor-mediated endocytosis</i>	123
Indications for gastrointestinal gene therapy	123
<i>Gene therapy for inflammatory disorders of the bowel</i>	124
Gene transfer to the salivary glands	125
<i>Potential clinical applications of salivary gene therapy</i>	125
Hematology	126
Hemophilias	126
<i>Gene therapy of hemophilia</i>	126
<i>Hemophilia A</i>	127
<i>Hemophilia B</i>	128
<i>Concluding remarks about gene therapy of hemophilias</i>	128
Hemoglobinopathies.....	129
<i>Stem cell-based gene therapy and RNAi for sickle cell disease</i>	129
<i>Gene therapy for β-thalassemia</i>	130
<i>Gene therapy of Fanconi's anemia</i>	131
Acquired hematopoietic disorders.....	132
<i>Chronic acquired anemias</i>	132
<i>Neutropenia</i>	132
<i>Thrombocytopenia</i>	133
Concluding remarks about gene therapy of hemoglobinopathies	134
Companies involved in gene therapy of hematological disorders	134
In utero gene therapy	135
Fetal gene transfer techniques.....	135
Animal models of fetal gene therapy	136
Potential applications of fetal gene therapy	136
<i>Fetal gene therapy for cystic fibrosis</i>	136
<i>Fetal intestinal gene therapy</i>	137
Hearing disorders	137
Potential of gene therapy	137
Vectors for gene therapy of hearing disorders.....	138
Auditory hair cell replacement and hearing improvement by gene therapy	138
Kidney diseases	139
End-stage renal disease.....	139
Methods of gene delivery to the kidney.....	139
<i>Gene transfer into kidney by adenoviral vectors</i>	140
<i>Non-viral gene transfer to the kidneys</i>	140
<i>Gene transfer into the glomerulus by HVJ-liposome</i>	140
<i>Bone marrow stem cells for renal disease</i>	140
<i>Mesangial cell therapy</i>	141
<i>Liposome-mediated gene transfer into the tubules</i>	141
<i>Gene transfer to tubules with cationic polymer polyethylenimine</i>	142
Gene therapy in animal experimental models of renal disease	142
Genetic manipulations of the embryonic kidney	142
Antisense intervention in glomerulonephritis	143
Gene therapy for renal fibrosis	143
Use of genetically engineered cells for uremia due to renal failure.....	143
Concluding remarks	144
Liver disorders	144
Techniques of gene delivery to liver	145
<i>Direct injection of DNA into liver</i>	145
<i>Local gene delivery by isolated organ perfusion</i>	145
<i>Liposome-mediated direct gene transfer</i>	146
<i>Retroviral vector for gene transfer to liver</i>	146
<i>Adenoviral vectors for gene transfer to liver</i>	146
<i>Receptor-mediated approach</i>	146
Cell therapy for liver disorders.....	147
<i>Transplantation of genetically modified hepatocytes</i>	147
<i>Genetically modified hematopoietic stem cells</i>	147
<i>Gene therapy by ex vivo transduced liver progenitor cells</i>	147
Gene therapy of genetic diseases affecting the liver	148
<i>Crigler-Najjar syndrome</i>	148
<i>Hereditary tyrosinemia type I (HT1)</i>	148
<i>Hereditary tyrosinemia type 3</i>	148
Gene therapy of acquired diseases affecting the liver	149
<i>Cirrhosis of liver</i>	149
Ophthalmic disorders	149
Introduction to gene therapy of ophthalmic disorders	149

Degenerative retinal disorders	150
<i>Age-related macular degeneration</i>	150
<i>Inherited retinal degenerations</i>	152
Inherited disorders affecting vision.....	153
<i>Gene therapy for color blindness</i>	153
<i>Leber congenital amaurosis</i>	153
<i>Retinitis pigmentosa</i>	154
<i>Stargardt disease</i>	154
<i>Usher syndrome</i>	155
<i>X-linked juvenile retinoschisis</i>	155
Proliferative retinopathies	155
Methods of gene transfer to retinal cells	156
<i>DNA nanoparticles for nonviral gene transfer to the eye</i>	157
Prevention of complications associated with eye surgery	157
<i>Prevention of proliferative retinopathy by gene therapy</i>	157
<i>DNA nanoparticles for gene therapy of retinal degenerative disorders</i>	157
<i>Posterior capsule opacification after cataract surgery</i>	158
Autoimmune uveitis	158
Retinal ischemic injury.....	158
Corneal disorders.....	159
Glaucoma	160
Disorders of hearing	160
Gene therapy for hearing loss.....	160
Organ transplantation.....	161
Introduction	161
Veto cells and transplant tolerance.....	161
Gene therapy for prolonging allograft survival	162
Gene therapy in lung transplantation.....	162
Role of gene therapy in liver transplantation	162
Gene therapy in kidney transplantation.....	163
Pulmonary disorders.....	163
Techniques of gene delivery to the lungs	163
<i>Adenoviral vectors</i>	164
<i>Non-viral vectors</i>	164
<i>Aerosolization as an aid to gene transfer to lungs</i>	165
Cystic fibrosis	165
<i>Genetics and clinical features</i>	165
<i>Gene therapy for CF</i>	166
<i>CFTR gene transfer in CF</i>	166
<i>Concluding remarks about gene therapy of CF</i>	167
Miscellaneous pulmonary disorders	168
<i>Gene therapy for pulmonary arterial hypertension</i>	168
<i>Gene therapy for bleomycin-induced pulmonary fibrosis</i>	169
<i>Pulmonary complications of α1-antitrypsin deficiency</i>	169
<i>Gene therapy for asthma</i>	170
<i>Gene therapy for adult respiratory distress syndrome</i>	170
<i>Gene therapy for lung injury</i>	171
<i>Gene therapy for bronchopulmonary dysplasia</i>	171
Concluding remarks about gene therapy of lungs.....	171
Companies involved in pulmonary gene therapy	172
Skin and soft tissue disorders.....	172
Gene transfer to the skin	172
<i>Electroporation for transdermal delivery of DNA vaccines</i>	173
<i>Ultrasound and topical gene therapy</i>	173
Gene therapy in skin disorders	173
<i>Gene therapy of hair loss</i>	173
<i>Gene therapy for xeroderma pigmentosa</i>	174
<i>Gene therapy for lamellar ichthyosis</i>	174
<i>Gene therapy for epidermolysis bullosa</i>	175
Gene transfer techniques for wound healing	175
Urogenital disorders	176
Gene therapy for urinary tract dysfunction	176
Gene therapy for erectile dysfunction	176
<i>NOS gene transfer for erectile dysfunction</i>	176
<i>Clinical trial of hMaxi-K Gene transfer in erectile dysfunction</i>	177
<i>Gene therapy for erectile dysfunction due to nerve injury</i>	177
<i>Concluding remarks on gene therapy for erectile dysfunction</i>	177
Veterinary gene therapy	177
Gene therapy for mucopolysaccharidosis VII in dogs	178
Gene therapy to increase disease resistance.....	178
Gene therapy for infections	178

Gene therapy for chronic anemia	179
Gene therapy for endocrine disorders	179
Gene therapy for arthritis.....	179
Cancer gene therapy	180
<i>Brain tumors in cats and dogs</i>	180
<i>Breast cancer in dogs</i>	181
<i>Canine hemangiosarcoma</i>	181
<i>Canine melanoma</i>	182
<i>Canine soft tissue sarcoma</i>	182
Melanoma in horses	182
4. Gene Therapy of Genetic Disorders	183
Introduction	183
Primary immunodeficiency disorders.....	184
Severe combined immune deficiency	185
Chronic granulomatous disease	187
Wiskott-Aldrich syndrome	187
Purine nucleoside phosphorylase deficiency	188
Major histocompatibility class II deficiency	188
Future prospects of gene therapy of inherited immunodeficiencies	189
Metabolic disorders.....	189
Adrenoleukodystrophy	190
Canavan disease.....	190
Lesch-Nyhan syndrome	191
Ornithine transcarbamylase deficiency.....	191
Phenylketonuria.....	192
Porphyrias	192
Tetrahydrobiopterin deficiency.....	193
Lysosomal storage disorders.	193
Batten disease.....	194
Fabry's disease.....	195
Farber's disease.....	195
Gaucher disease	195
<i>Animals models of Gaucher's disease</i>	196
<i>Gene therapy of Gaucher's disease</i>	196
Hunter syndrome.....	197
Combination of cell and gene therapy for Krabbe's disease.....	197
Metachromatic leukodystrophy	198
Mucopolysaccharidosis type 1 (Hurler syndrome)	198
Niemann-Pick type A disease.....	199
Pompe disease	199
Sanfilippo A syndrome.....	200
Sly syndrome	200
Tay-Sachs disease	200
Future prospects of gene therapy of lysosomal storage disorders	201
Trinucleotide repeat disorders.....	201
Muscular dystrophies.....	201
Duchenne muscular dystrophy (DMD).....	201
<i>Animal models for gene therapy of DMD</i>	202
<i>Types of dystrophin constructs</i>	202
<i>Antisense approach to DMD</i>	203
<i>Post-transcriptional modulation of gene expression in DMD</i>	204
<i>Myoblast-based gene transfer in DMD</i>	204
<i>Plasmid-mediated gene therapy</i>	204
<i>Liposome-mediated gene transfer</i>	205
<i>Viral vectors for DMD</i>	205
<i>Routes of administration of gene therapy in DMD</i>	206
<i>Conclusions and future prospects of gene therapy of DMD</i>	206
Limb-girdle muscular dystrophy	207
Myotonic dystrophy	208
Spinal muscular atrophy	208
Antisense gene therapy of SMA	208
Hereditary neuropathies	209
Charcot-Marie-Tooth disease	209
Hereditary axonal neuropathies of the peripheral nerves.....	209
Gene therapy of mitochondrial disorders	210
Companies involved in gene therapy of genetic disorders	210
5. Gene Therapy of Cancer.....	213
Strategies for cancer gene therapy.....	213
Direct gene delivery to the tumor	214

Injection into tumor	214
<i>Direct injection of adenoviral vectors</i>	214
<i>Direct injection of a plasmid DNA-liposome complex</i>	215
<i>A polymer approach to local gene therapy for cancer</i>	215
Electroporation for cancer gene therapy.....	215
Control of gene expression in tumor by local heat	216
Radiation-guided gene therapy of cancer	216
Nanoparticles to facilitate combination of hyperthermia and gene therapy.....	217
Cell-based cancer gene therapy	217
Adoptive cell therapy	217
Cytokine gene therapy.....	218
Genetic modification of human hematopoietic stem cells.....	221
Immunogene therapy	221
Cancer vaccines	222
Genetically modified cancer cell vaccines	222
<i>GVAX cancer vaccines</i>	222
<i>Genetically modified dendritic cells</i>	223
Nucleic acid-based cancer vaccines	223
<i>DNA cancer vaccines</i>	224
<i>RNA vaccines</i>	224
Viral vector-based cancer vaccines	224
<i>Intradermal delivery of cancer vaccines by Ad vectors</i>	225
Future prospects of cancer vaccines	225
Companies involved in nucleic acid-based cancer vaccines	226
Monoclonal antibody gene transfer for cancer	227
Transfer and expression of intracellular adhesion-1 molecules	227
Other gene-based techniques of immunotherapy of cancer	227
Fas (Apo-1).....	227
Chemokines	228
Major Histocompatibility Complex (MHC) Class I	228
IGF (Insulin-Like Growth Factor).....	228
Inhibition of immunosuppressive function in cancer	229
Delivery of toxic genes to tumor cells for eradication	229
Gene-directed enzyme prodrug therapy	229
Combination of gene therapy with radiotherapy	230
Correction of genetic defects in cancer cells	230
Targeted gene therapy for cancer	231
Bacteria as novel anticancer gene vectors	231
Cancer-specific gene expression	231
Cancer-specific transcription.....	231
Delivery of retroviral particles hitchhiking on T cells	232
Electrogene and electrochemotherapy	232
Epidermal growth factor-mediated DNA delivery	232
Gene-based targeted drug delivery to tumors.....	233
Gene expression in hypoxic tumor cells	233
Genetically modified T cells for targeting tumors.....	234
Genetically engineered stem cells for targeting tumors	234
Hematopoietic stem cells for targeted cancer gene therapy	235
Immunolipoplex for delivery of p53 gene	235
Nanomagnets for targeted cell-based cancer gene therapy.....	236
Nanoparticles for targeted site-specific delivery of anticancer genes	236
Targeted cancer therapy using a dendrimer-based synthetic vector.....	237
Tumor-targeted gene therapy by receptor-mediated endocytosis	237
Virus-mediated oncolysis	237
<i>Targeted cancer treatments based on oncolytic viruses</i>	237
<i>Oncolytic HSV</i>	238
<i>Oncolytic adenoviruses</i>	238
<i>Oncolytic vesicular stomatitis virus</i>	240
<i>Oncolytic paramyxovirus</i>	240
<i>Oncolytic vaccinia virus</i>	240
<i>Cancer terminator virus</i>	240
<i>Cytokine-induced killer cells for delivery of an oncolytic virus</i>	241
<i>Monitoring of viral-mediated oncolysis by PET</i>	241
<i>Oncolytic gene therapy</i>	242
<i>Companies developing oncolytic viruses</i>	242
Apoptotic approach to improve cancer gene therapy	243
Tumor suppressor gene therapy	243
P53 gene therapy.....	244
BRIT1 gene therapy	244
Nitric oxide-based cancer gene therapy	244
Nitric oxide synthase II DNA injection.....	244

Gene therapy for radiosensitization of cancer	244
Gene therapy of cancer of selected organs	245
Gene therapy for bladder cancer	245
Gene therapy for glioblastoma multiforme.	246
<i>Targeted adenoviral vectors</i>	247
<i>Genetically engineered MSCs for gene delivery to intracranial gliomas</i>	247
<i>Targeting normal brain cells with an AAV vector encoding interferon-β</i>	247
<i>Viral oncolysis of brain tumors</i>	248
<i>Autophagy induced by conditionally replicating adenoviruses</i>	248
<i>Oncolytic virus targeted to brain tumor stem cells</i>	249
<i>Antiangiogenic gene therapy</i>	249
<i>Baculovirus vector for diphtheria toxin gene therapy</i>	250
<i>Intravenous gene delivery with nanoparticles into brain tumors</i>	250
<i>Gene therapy targeting hepatocyte growth factor</i>	250
<i>RNAi gene therapy of brain cancer</i>	250
<i>Ligand-directed delivery of dsRNA molecules targeted to EGFR</i>	251
Gene therapy for breast cancer.....	251
<i>Intratumoral injection of Ad5CMV-p53 (Advexin)</i>	252
<i>Gene vaccine for breast cancer</i>	252
<i>Recombinant adenoviral ErbB-2/neu vaccine</i>	252
Gene Therapy for ovarian cancer	253
Gene therapy for malignant melanoma	254
Gene therapy of lung cancer.....	256
<i>Intravenous nanoparticle formulation for delivery of FUS1 gene</i>	256
<i>Aerosol gene delivery for lung cancer</i>	256
Gene therapy for cancer of prostate	256
<i>Experimental studies</i>	257
<i>Nanoparticle-based gene therapy for prostate cancer</i>	257
<i>Tumor suppressor gene therapy in prostate cancer</i>	257
<i>Vaccine for prostate cancer</i>	258
<i>Clinical trials</i>	258
Gene therapy of head and neck cancer	258
<i>Adenoviral vector based P53 gene therapy</i>	259
Gene therapy of pancreatic cancer	259
<i>Rexin-G™ for targeted gene delivery in cancer</i>	259
<i>Targeted Expression of BikDD gene</i>	260
<i>Concluding remarks on gene therapy of pancreatic cancer</i>	260
Cancer gene therapy companies	260
6. Gene Therapy of Neurological Disorders	265
Indications	265
Gene transfer techniques for the nervous system.....	266
Methods of gene transfer to the nervous system.....	266
Ideal vector for gene therapy of neurological disorders	266
Promoters of gene transfer.....	266
Lentivirus-mediated gene transfer to the CNS	267
AAV vector mediated gene therapy for neurogenetic disorders.....	267
Gene transfer to the CNS using recombinant SV40-derived vectors	268
Routes of delivery of genes to the CNS	268
<i>Direct injection into CNS</i>	268
<i>Introduction of the genes into cerebral circulation</i>	269
<i>Introduction of genes into cerebrospinal fluid</i>	269
<i>Intravenous administration of vectors</i>	269
<i>Delivery of gene therapy to the peripheral nervous system</i>	270
Cell-mediated gene therapy of neurological disorders	270
<i>Neuronal cells</i>	270
<i>Neural stem cells and progenitor cells</i>	270
<i>Astrocytes</i>	270
<i>Cerebral endothelial cells</i>	271
<i>Implantation of genetically modified encapsulated cells into the brain</i>	271
Gene therapy of neurodegenerative disorders	271
Gene therapy for Parkinson disease.....	271
Rationale	272
<i>Techniques of gene therapy for PD</i>	273
<i>Delivery of neurotrophic factors by gene therapy</i>	276
<i>Delivery of parkin gene</i>	277
<i>Introduction of functional genes into the brain of patients with PD</i>	277
<i>Nanoparticle-based gene therapy for PD</i>	277
<i>Mitochondrial gene therapy for PD</i>	277
<i>RNAi approach to PD</i>	278
<i>Prospects of gene therapy for PD</i>	278

<i>Companies developing gene therapy for PD</i>	279
Gene therapy for Alzheimer disease	280
<i>Rationale</i>	280
<i>NGF gene therapy for AD</i>	280
<i>Nepriylsin gene therapy</i>	281
<i>Targeting plasminogen activator inhibitor type-1 gene</i>	282
<i>Gene vaccination</i>	282
<i>Combination of gene therapy with other treatments for AD</i>	282
Gene therapy of Huntington disease.....	282
<i>Encapsulated genetically engineered cellular implants</i>	282
<i>Viral vector mediated administration of neurotrophic factors</i>	283
<i>RNAi gene therapy</i>	283
Gene therapy of amyotrophic lateral sclerosis.....	283
<i>Rationale</i>	283
<i>Technique of gene therapy of ALS</i>	283
Gene therapy of cerebrovascular diseases.....	285
Preclinical research in gene therapy for cerebrovascular disease	285
Animal models of stroke relevant to gene therapy.....	285
<i>Transgenic mice as models for stroke</i>	285
<i>Animal models for gene therapy of arteriovenous malformations</i>	286
Gene transfer to cerebral blood vessels	286
Gene therapy for vasospasm following subarachnoid hemorrhage	287
<i>NOS gene therapy for cerebral vasospasm</i>	288
Gene therapy for stroke.....	288
<i>Gene therapy for stroke using neurotrophic factors</i>	289
<i>Gene therapy of strokes with a genetic component</i>	290
<i>Gene therapy for intracranial aneurysms</i>	290
Concluding remarks about gene therapy for stroke.....	290
Gene therapy of injuries to the nervous system.....	291
Traumatic brain injury	291
Spinal cord injury	291
Gene therapy of epilepsy	292
Gene therapy for control of seizures	292
Gene therapy for neuroprotection in epilepsy	293
Gene therapy for genetic forms of epilepsy	294
Gene therapy for multiple sclerosis	294
Gene therapy for relief of pain	294
Rationale of gene therapy for pain	294
Vectors for gene therapy of pain	295
Methods of gene delivery for pain	295
<i>Endogenous analgesic production for cranial neuralgias</i>	296
<i>Gene delivery by intrathecal route</i>	296
<i>Gene transfer for delivery of analgesics to the spinal nerve roots</i>	297
<i>Gene therapy of peripheral neuropathic pain</i>	298
<i>Gene transfer by injections into the brain substance</i>	298
Targets for gene therapy of pain	298
<i>Zinc finger DNA-binding protein therapeutic for chronic pain</i>	298
<i>Gene therapy for producing enkephalin to block pain signals</i>	299
<i>Targeting nuclear factor-κB</i>	299
<i>Gene therapy targeted to neuroimmune component of chronic pain</i>	299
Potential applications of gene therapy for management of pain.....	300
Concluding remarks on gene therapy for pain	300
Gene therapy for psychiatric disorders	301
Gene therapy for depression	301
Gene therapy for enhancing cognition after stress	302
Companies involved in gene therapy of neurological disorders.....	302

7. Gene Therapy of Cardiovascular Disorders **303**

Introduction	303
Techniques of gene transfer to the cardiovascular system	303
Direct plasmid injection into the myocardium	304
Catheter-based systems for vector delivery.....	304
Ultrasound microbubbles for cardiovascular gene delivery	305
Vectors for cardiovascular gene therapy	305
<i>Adenoviral vectors for cardiovascular diseases</i>	305
<i>Plasmid DNA-based delivery in cardiovascular disorders</i>	305
<i>Intravenous rAAV vectors for targeted delivery to the heart</i>	306
Hypoxia-regulated gene therapy for myocardial ischemia.....	306
Angiogenesis and gene therapy of ischemic disorders	306
Therapeutic angiogenesis vs vascular growth factor therapy.....	307
Gene painting for delivery of targeted gene therapy to the heart	307

Gene delivery to vascular endothelium	308
Targeted plasmid DNA delivery to the cardiovascular system with nanoparticles	308
Vascular stents for gene delivery	308
Gene therapy for genetic cardiovascular disorders	309
Genetic disorders predisposing to atherosclerosis.....	309
Familial hypercholesterolemia (FH)	309
Apolipoprotein E (apoE) deficiency	311
Hypertension.....	311
Genetic factors for myocardial infarction	312
Acquired cardiovascular diseases	312
Coronary artery disease with angina pectoris.....	312
<i>Ad5FGF-4</i>	312
Ischemic heart disease with myocardial infarction	313
<i>Myocardial repair with IGF-1 therapy</i>	314
<i>Metalloproteinase-2 inhibitor gene therapy</i>	314
Congestive heart failure.....	315
<i>Rationale of gene therapy in CHF</i>	315
<i>-ARKct gene therapy</i>	315
<i>Intracoronary adenovirus-mediated gene therapy for CHF</i>	316
<i>AAV-mediated gene transfer for CHF</i>	316
<i>AngioCell gene therapy for CHF</i>	316
<i>nNOS gene transfer in CHF</i>	317
Cardiomyopathies	317
Cardiac conduction disturbances	317
<i>Gene transfer approaches for biological pacemakers</i>	317
<i>Genetically engineered biological pacemakers</i>	318
Gene therapy and heart transplantation.....	319
Peripheral arterial disease	319
Incidence and clinical features	319
Current management	320
Gene therapy for peripheral arterial disease	320
<i>Angiogenesis by gene therapy</i>	320
<i>HIF-1α gene therapy for peripheral arterial disease</i>	320
<i>HGF gene therapy for peripheral arterial disease</i>	321
Ischemic neuropathy secondary to peripheral arterial disease.....	321
Prevention of restenosis after angioplasty	321
<i>Antisense approaches</i>	322
<i>Gene therapy to prevent restenosis after angioplasty</i>	322
<i>Techniques of gene therapy for restenosis</i>	323
<i>NOS gene therapy for restenosis</i>	324
<i>hTIMP-1 gene therapy to prevent intimal hyperplasia</i>	325
Maintaining vascular patency after surgery	325
Companies involved in gene therapy of cardiovascular diseases	325
Future prospects of gene therapy of cardiovascular disorders	326
8. Gene therapy of viral infections	329
Introduction	329
Acquired Immunodeficiency Syndrome (AIDS).....	329
Current management of AIDS.....	329
Gene therapy strategies in HIV/AIDS.....	330
<i>HIV/AIDS vaccines</i>	330
<i>Insertion of protective genes into target cells</i>	331
Cell/gene therapies for HIV/AIDS.....	332
<i>Transplantation of genetically modified T-cells</i>	332
<i>Transplantation of genetically modified hematopoietic cells</i>	332
<i>Anti-HIV ribozyme delivered in hematopoietic progenitor cells</i>	333
Inhibition of HIV-1 replication by lentiviral vectors	333
<i>VRX496</i>	333
Intracellular immunization.....	334
Engineered cellular proteins such as soluble CD4s.....	334
Intracellular antibodies	334
Anti-rev single chain antibody fragment.....	334
Use of genes to chemosensitize HIV-1 infected cells.....	335
Autocrine interferon (INF)- β production by somatic cell gene therapy	335
Antisense approaches to AIDS	335
<i>RNA decoys</i>	335
<i>Antisense oligodeoxynucleotides</i>	335
<i>RNA decoys</i>	336
<i>Ribozymes</i>	336
RNAi applications in HIV/AIDS	337
<i>siRNA-directed inhibition of HIV-1 infection</i>	337

<i>Role of the nef gene during HIV-1 infection and RNAi</i>	337
<i>Bispecific siRNA constructs</i>	338
<i>Targeting CXCR4 with siRNAs</i>	338
<i>Targeting CCR5 with siRNAs</i>	338
Companies involved in developing gene therapy for HIV/AIDS	339
Conclusions regarding gene therapy of HIV/AIDS	340
Genetic vaccines for other viral infections	340
Cytomegalic virus infections	340
Viral hepatitis	341
<i>Vaccine for hepatitis B virus</i>	341
<i>Vaccine for hepatitis C virus</i>	342
Vaccine for herpes simplex virus	342
DNA vaccine against rabies	342
DNA vaccine for Ebola	343
Vaccines for avian influenza	343
<i>Future prospects of DNA vaccines for avian influenza</i>	344
<i>Human trial of a DNA vaccine for avian influenza</i>	345
Companies developing genetic vaccines for infections other than AIDS	345
9. Research, Development and Future of Gene Therapy	347
Basic research in gene therapy	347
R & D in gene therapy	347
Animal models of human diseases for gene therapy research	348
<i>Lentiviral transgenesis</i>	348
Financing research and development	348
Role of the NIH in gene therapy research	348
National Gene Vector Laboratories	348
Financing by the industry	349
Clinical trials in gene therapy	349
Clinical trials worldwide	349
<i>Clinical trials in cancer gene therapy</i>	350
<i>Clinical trials in cardiovascular gene therapy</i>	350
<i>Clinical trials in inherited monogenic diseases</i>	350
<i>Clinical trials for other indications</i>	351
Clinical trials in the US	351
Vectors used in gene therapy clinical trials	352
Future prospects for the gene therapy	353
How to improve gene therapy	353
Promising areas of application of gene therapy	354
<i>Neurological disorders</i>	354
<i>Gene therapy of cardiovascular disorders</i>	355
<i>Cancer gene therapy</i>	356
Personalized gene therapy	356
10. Regulatory, Safety and Ethical Issues of Gene Therapy	359
Regulation of gene therapy in the United States	359
US Federal guidelines for research involving recombinant DNA molecules	359
Regulation of gene therapy in US	359
Office of Biotechnology Activities	359
Implantation of genetically manipulated cells	360
Clinical trials in gene therapy	360
Cell and gene therapy INDs placed on hold by the FDA	360
Regulation of gene therapy in Germany	361
Preclinical research	361
Clinical Trials	361
Marketing authorization	362
Regulation of gene therapy in the United Kingdom	362
Regulation of gene therapy in France	363
Regulation of gene therapy in the Netherlands	363
Regulation of gene therapy in Australia	364
Regulation of gene therapy in Japan	365
Regulation of gene therapy in China	365
Safety issues of gene transfer	365
Adverse effects of retroviral vectors	365
<i>Insertional mutagenesis</i>	365
Adverse effects of HSV vectors	366
<i>Neurotoxicity of HSV vectors</i>	366
<i>Hepatotoxicity of HSV-tk/ganciclovir approach</i>	366
Adverse effects of adenoviral vectors	366
<i>Inflammatory effects of adenoviruses in lungs</i>	367
<i>Inflammatory effects involving the liver</i>	367

<i>Induction of immune response by adenoviral vectors</i>	367
<i>Impairment of adrenocortical steroidogenesis</i>	368
<i>Adverse effects of AAV vectors</i>	368
Toxicity associated with cationic lipid-mediated gene transfer	368
Toxicity of lipopolysaccharides	369
Potential side effects of RNAi gene therapy.....	369
Role of molecular diagnostics in safety of gene therapy	369
Quality control of vectors	370
<i>Testing for retroviruses</i>	370
<i>Adenoviral vectors</i>	370
<i>Replication competent viruses</i>	370
Genetic characteristics of viral vectors	371
Concluding remarks about safety of viral vectors	371
Ethical aspects of gene therapy	371
The lay consumer's view of somatic gene therapy ethics	372
Ethical aspects of clinical trials.....	372
Ethical aspects of germline gene therapy	373
Germline gene therapy for genetic enhancement	373
Athletic enhancement by genetic engineering	373
<i>Gene doping in sports</i>	374
<i>Gene transfer methods used for enhancing physical performance</i>	374
<i>Adverse effect of genetic engineering</i>	376
<i>Problems in detecting genetic manipulations in athletes</i>	376
<i>Ethical dilemma</i>	376
11. Markets for Gene Therapy	379
Introduction	379
Gene therapy markets in various regions of the world	379
Gene therapy markets according to therapeutic areas	380
Cancer gene therapy market	380
Markets for gene therapy of genetic disorders	380
Markets for DNA vaccines	381
DNA vaccines markets according to technologies	381
DNA vaccines markets according to therapeutic indications	381
DNA vaccines markets according to geographical areas	382
Competing treatments	382
Antisense.....	383
RNAi.....	383
Cell therapy	383
Strategies for developing gene therapy markets	383
<i>Collaboration with pharmaceutical companies</i>	384
<i>Collaboration with companies developing cell-based therapies</i>	384
<i>Overcoming obstructions to the development of gene therapy</i>	384
<i>Collaboration with academic gene therapy centers</i>	384
<i>Developing safer and cost-effective gene medicines</i>	384
Unmet needs in gene therapy	385
Promising areas for the development of gene therapy	385
12. References	387

Tables

Table 1-1: Landmarks in development of gene therapy.....	21
Table 2-1: Classification of methods of gene therapy.....	31
Table 2-2: A comparison of various physical methods of gene transfer	33
Table 2-3: Experimental applications of gene transfer by electroporation	35
Table 2-4: An overview of characteristics of commonly used viral vectors	45
Table 2-5: Companies using viral vectors.....	57
Table 2-6: Companies using nonviral vectors	74
Table 2-7: Target organs for non-viral gene therapy methods.	76
Table 2-8: Potential routes for administration of DNA	83
Table 2-9: Companies with gene delivery devices/ technologies.....	84
Table 2-10: Strategies for targeted gene therapy	85
Table 2-11: In vivo animal experimental studies of gene delivery with polymeric systems	87
Table 2-12: Approaches to controlling gene expression in gene therapy	88
Table 2-13: Companies with regulated / targeted gene therapy and special techniques	90
Table 2-14: Potential applications of human germline genome modification	92
Table 2-15: Applications of molecular diagnostics in gene therapy	104
Table 2-16: Advantages of gene therapy compared with protein therapy	106

Table 3-1: Experimental approaches to gene therapy of rheumatoid arthritis	110
Table 3-2: Gene therapy strategies for osteoarthritis.....	112
Table 3-3: Cell and gene therapy approaches for type 1 diabetes mellitus.....	117
Table 3-4: Indications for gastrointestinal gene therapy	124
Table 3-5: Hematological disorders that can be potentially treated by gene therapy.....	126
Table 3-6: Companies involved in gene therapy of hematological disorders.....	134
Table 3-7: Techniques of gene transfer to the kidneys.....	139
Table 3-8: Gene therapy in animal experimental models of renal disease.....	142
Table 3-9: Applications of gene therapy in ophthalmological disorders.....	150
Table 3-10: Strategies for gene delivery to the lungs	163
Table 3-11: Companies developing gene therapy for pulmonary disorders	172
Table 4-1: Genetic disorders that are being investigated for gene therapy	183
Table 4-2: X-linked immunodeficiency disorders	185
Table 4-3: Examples of inherited metabolic disorders amenable to gene therapy	189
Table 4-4: Gene therapy approaches to Duchenne muscular dystrophy	202
Table 4-5: Companies involved in gene therapy of genetic/metabolic disorders	210
Table 5-1: Strategies for cancer gene therapy	213
Table 5-2: Cell-based gene therapy for cancer	217
Table 5-3: Companies with nucleic acids/genetically modified cell cancer vaccines	226
Table 5-4: Enzyme/prodrug combinations employed in suicide gene therapy.....	229
Table 5-5: Mutation compensation strategies used clinically	230
Table 5-6: Companies developing oncolytic viruses	242
Table 5-7: Strategies for gene therapy of malignant brain tumors.....	246
Table 5-8: Clinical trials of gene therapy in ovarian cancer.....	253
Table 5-9: Gene therapy for malignant melanoma	254
Table 5-10: Clinical trials in gene therapy for prostate cancer.....	258
Table 5-11: Companies involved in cancer gene therapy.....	260
Table 6-1: Example of potential indications for gene therapy of neurologic disorder	265
Table 6-2: Methods of gene transfer as applied to neurologic disorders	266
Table 6-3: Gene therapy techniques applicable to Parkinson disease	272
Table 6-4: Companies developing gene therapy for Parkinson's disease.....	279
Table 6-5: Gene transfer in animal models of carotid artery restenosis.....	286
Table 6-6: Gene therapy strategies for vasospasm.....	287
Table 6-7: Neuroprotective gene therapy in animal stroke models	288
Table 6-8: Experimental gene therapy approaches for relief of pain	295
Table 6-9: Companies involved in gene therapy of neurological disorders	302
Table 7-1: Cardiovascular disorders for which gene therapy is being considered.	303
Table 7-2: Catheter-based systems for vector delivery to the cardiovascular system.....	304
Table 7-3: Companies involved in gene therapy of cardiovascular diseases	326
Table 8-1: Strategies for gene therapy of AIDS	330
Table 8-2: Companies involved in developing gene therapy for HIV/AIDS	339
Table 8-3: Companies developing genetic vaccines for infections other than AIDS	345
Table 9-1: Clinical trials of gene therapy in the US according to applications	351
Table 9-2: Potential future applications of gene therapy in disorders of the nervous system	354
Table 10-1: Genes that may be used for performance enhancement	374
Table 11-1: Gene therapy market according to regions/countries – 2009 to 2019	379
Table 11-2: Gene therapy markets according to therapeutic areas – 2009 to 2019.....	380
Table 11-3: Cancer gene therapy market according to type of cancer - 2009 to 2019.....	380
Table 11-4: Gene therapy market for selected genetic disorders - 2009 to 2019.....	381
Table 11-5: DNA vaccines markets according to technologies - 2009 to 2019.....	381
Table 11-6: DNA vaccines markets according to therapeutic indications - 2009 to 2019.....	382
Table 11-7: DNA vaccines markets according to geographical areas - 2009 to 2019.....	382

Figures

Figure 1-1: Relation of gene therapy to other biotechnologies	23
Figure 1-2: Relationship of DNA, RNA and protein in the cell	27
Figure 2-1: Ex vivo and in vivo techniques of gene therapy.....	32
Figure 2-2: Structure of the Helios gene gun.....	37
Figure 2-3: Cochleate-mediated gene therapy	67
Figure 2-4: Bacteria plus nanoparticles for drug delivery into cells	72
Figure 2-5: Schematic of suppression of gene expression by RNAi	102
Figure 6-1: Effect of tyrosine hydroxylase gene delivery on dopamine levels	273
Figure 6-2: Role of cell and gene therapy in stroke according to pathology and stage.....	290
Figure 9-1: Product development cycle in gene therapy.....	347
Figure 9-2: Proportions of therapeutic areas in clinical trials of gene therapy in the US.....	352
Figure 9-3: Proportions of various vectors used in gene therapy trials	352
Figure 11-1: Unmet needs in gene therapy	385