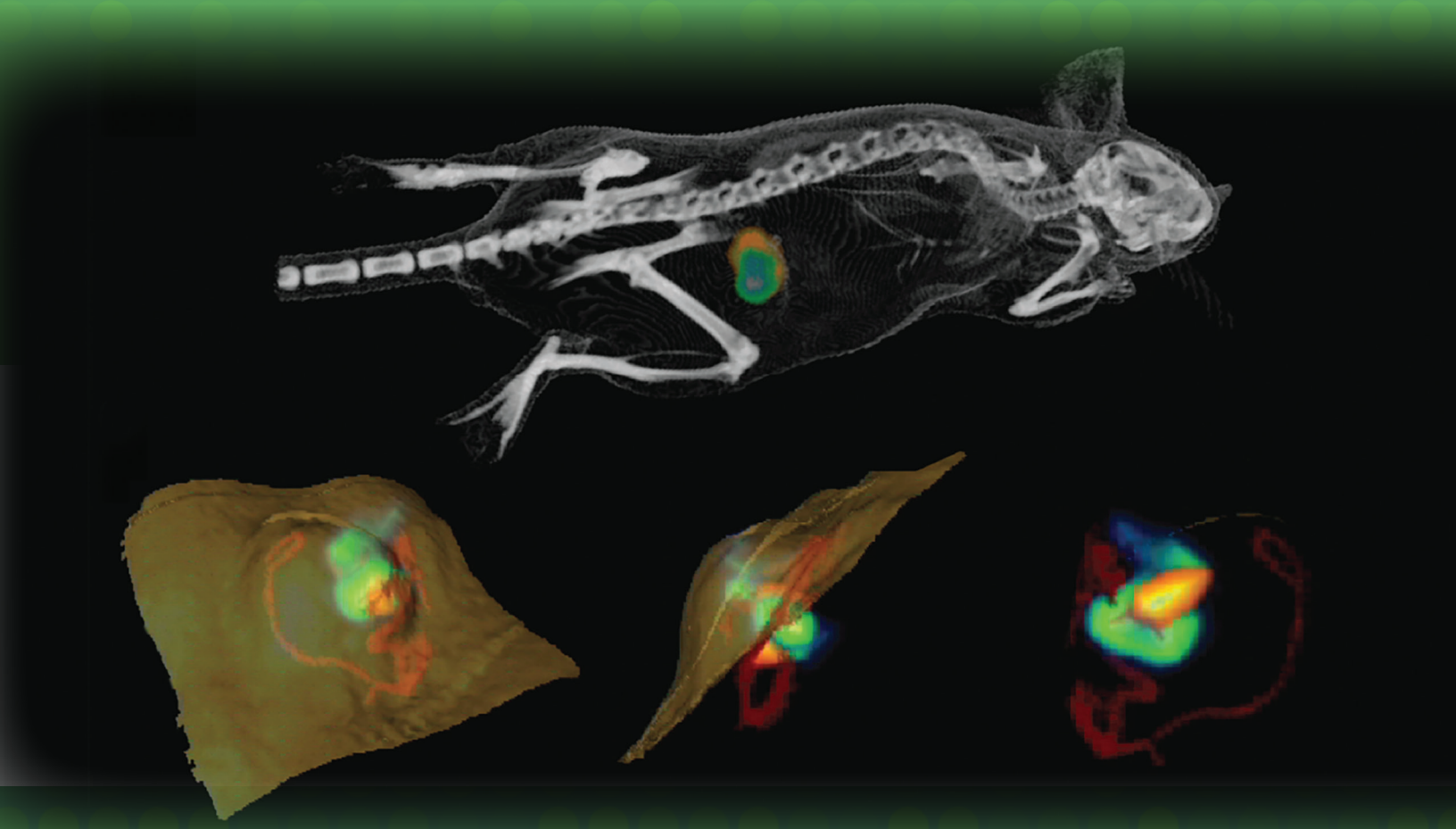
FOURTH EDITION

GENE AND CELL THERAPY

Therapeutic Mechanisms and Strategies



EDITED BY

Nancy Smyth Templeton

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Foreword: Gene and Cell Therapy Matures to the Clinics

Gene therapy has finally gained official recognition in Western countries following approval of the first gene medicine for commercial use in 2012. Glybera®, an adenovirus-based product for the treatment of lipoprotein lipase deficiency, was approved in the European Union after a long regulatory process [1]. This was a significant milestone in the maturation of this new technology and should encourage further development and clinical testing of therapeutic approaches based on gene transfer technologies. Worldwide, the first gene therapy products were approved in China for the treatment of cancer in 2003 and 2005 [2]. However, these adenoviral products (Gendicine and Oncorine) have so far not been approved for commercial use in Western countries.

Gene therapy has had a long journey to the clinics. The road has been shadowed by some widely publicized setbacks, which historically are very typical for new groundbreaking technologies in medicine. Several examples show that today's successful therapies, such as bone marrow transplantation, organ transplantation, cancer chemotherapy and monoclonal antibodies, have had a similar path from the first discoveries and proof-of-principle studies to the current status of their use in clinical medicine.

During the last few years, remarkable progress has been made in gene and cell therapy. Positive proof-of-principle results have been obtained for several diseases, such as SCID-X1 [3,4], ADA-SCID [5], Leber's congenital amaurosis [6,7], adrenoleukodystrophy [8], Wiskott–Aldrich syndrome [9], hemophilia IX [10], β-thalassemia [11], leukemia [12], malignant glioblastoma [13] and some other tumors [14]. Thus, it is expected that several new gene therapy products will enter the clinical arena in the not-so-distant future. In cell therapy, iPS cell technology has opened huge possibilities for cell therapy and regenerative medicine [15].

There are still significant challenges in gene and cell therapy, before these new approaches can enter main stream medicine. Significant safety issues have been reported in some trials [3,4] and the need for better vectors, delivery techniques and treatment genes is widely recognized. Manufacturing large quantities of gene and cell therapy products fulfilling multiple regulatory demands is still very challenging and one of the most significant bottlenecks in clinical translation of new promising therapies. Entirely new technologies to regulate expression of endogenous genes have been developed, and RNAi, siRNA, miRNA and gene targeting technologies have entered the field with great potential for successful therapeutic use. Regulating transgene, controlling potential immunological reactions against vectors and transgenes and avoiding genotoxicity with integrating vectors and targeting therapies to specific cell types in vivo are among the major challenges for the future [16].

In cell therapy, there is significant future potential for tissue regeneration and even replacement of damaged tissues with either various types of progenitor cells or even with iPS cells [15]. Notwithstanding significant challenges that are related to the potential malignant transformation and difficulties in directing stem cells and progenitor cells towards functional, specific cell types that would maintain their phenotype and functionality after delivery in vivo, it is likely that these technologies combined with advanced gene transfer techniques and small molecule effectors to control cell differentiation and growth can bring major advances to the field.

As in every new technology, teaching and educating next-generation scientists, physicians, government officials, who set rules for these therapies, and the general public require high-quality materials and textbooks in universities, colleges and other educational institutions. This textbook edited by Nancy Smyth Templeton is exactly what is needed to advance the fields of gene and cell therapy and for general education and further refinement of the technologies. Scientifically top-level, latest-stage chapters and overviews of the field are the best we can offer to new researchers, doctors and laypeople who want to learn the basics and get further insights in this new technology and its potential applications. With over a thousand pages describing the current gene transfer and vector technologies and the basics of cell therapy and clinical applications, this fourth edition of *Gene and Cell Therapy: Therapeutic Mechanisms and Strategies* gives an excellent, comprehensive basis to advance gene medicine and cell therapy towards successful future applications in medical practice.

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Preface

Our fourth edition of *Gene and Cell Therapy: Therapeutic Mechanisms and Strategies* provides extensive background and basic information, state-of-the-art technologies, important achievements, and lingering challenges in the fields of gene and cell therapies. Section I of this book covers delivery systems and therapeutic strategies. In Chapter 1, Anais Girard and Els Verhoeyen provide a thorough review of the design and applications of lentiviral vectors. The topics covered include lentiviral background; genomic structure and proteins; life cycle; nonhuman lentiviruses; HIV-1 lentiviral vectors (LVs) and their advantages; self-inactivating (SIN) LVs; first through fourth generation LVs; tropism; modifications for optimization including various targeting strategies; vector production for lab use and clinical trials, concentration, purification, and titration; and biosafety, applications and clinical trials. Florian Kreppel reviews adenovirus vector–mediated gene delivery in Chapter 2. Some of the topics covered include adenovirus biology and structure, cell entry, virus genome and life cycle, first through third generation vectors, vector–host interactions, barriers to delivery, immune activation, vector design, chemical capsid modifications, clinical trials, and remaining challenges.

Nicola Brunetti-Pierri and Philip Ng review the use of helper-dependent adenoviral vectors (HDAds) for gene therapy in Chapter 3. The topics covered include history and description of HDAds, viral infection and life cycle, mechanisms for cell entry and target cells, HDAd construction and production, infectious titer determination, duration of transgene expression, larger cloning capacity, vector serotypes, hybrid vectors, safety, preclinical studies, and clinical trials. Barrie Carter reviews adeno-associated virus and AAV vectors for gene delivery in Chapter 4. The topics covered include AAV background, life cycle and biology of AAV, epidemiology, cell entry and host tropism, particle and genome structure, replication, latency and persistence, integration, targeted integration, AAV permissivity, AAV vectors, vector production, complementation systems, replication competent or wild type AAV, purification, assays for AAV vectors, AAV serotypes, vector and vector DNA metabolism, cellular binding and trafficking, dual vectors, packing of oversized vectors, capsid modifications and targeting, host immune responses, toxicity, safety, preclinical studies, clinical trials for a wide variety of diseases and disorders, and future directions. In Chapter 5, Sheng Guo, Andrea McCart, and David Bartlett review vaccinia viral vectors. The topics covered include vaccinia viral (VV) biology and life cycle, VV strains, vector construction, in vitro and in vivo gene transfer, immune responses, in vivo pathogenicity and biodistribution, safety, preclinical studies, and clinical trials. Pavlos Msaouel and Evanthia Galanis review oncolytic measles virus–based delivery systems in Chapter 6. The topics covered include measles virus biology, historical perspective, cell entry and natural host tropism, genetic stability and safety of measles vaccine strains, genetic engineering of recombinant measles strains, retargeting of oncolytic measles virus strains, monitoring replication and spread, role of immunity in measles virotherapy, combination therapy and numerous anticancer approaches, appropriate animal models, preclinical studies and efficacy, clinical trials, and future directions. In Chapter 7, Kenneth Lundstrom reviews the field of alphaviruses including their characteristics and types, history, genetic engineering, life cycle, vectors and design, applications in neuroscience and gene therapy, and uses in clinical trials. Akseli Hemminki, Markus Vähä-Koskela, and Vincenzo Cerullo cover the field of oncolytic viruses for the treatment of cancer in Chapter 8. The topics covered include descriptions and applications of oncolytic viruses (adenovirus, herpes simplex virus, vaccinia virus, measles virus, Newcastle disease virus, and reovirus), historical to present-day review of oncolytic virotherapy, virus arming, clinical trials, and future directions. Kari Airenne and Seppo Ylä-Herttuala provide an extensive background and overview of baculovirus and baculoviral vectors in Chapter 9. This chapter covers historical to current uses of baculovirus and baculoviral vectors, their physical properties; transduction cell types, tissues, and organs; optimal transduction conditions, common cell entry pathways and intracellular transport, viral life cycle and replication; vector design, construction, and production; safety, therapeutic applications, and clinical trials; and future challenges.

Chapter 10, written by Yu-Chen Hu and colleagues, covers other current information and uses of baculoviral vectors including alternative cell entry pathways, novel vector design, applications particularly for bone and cartilage tissue engineering, safety profiles, and future directions. In Chapter 11, William Byrne and Mark Tangney review bacterial gene therapy vectors. The topics covered include history and rationale for the use of bacteria and bacterial vectors to treat cancer, bacterial gene delivery strategies, therapeutic strategies using bacterial vectors, safety, preclinical studies, clinical trials, and future directions. David Oupický et al. cover the topic of redox-responsive polymer-based delivery systems in Chapter 12, including their unique features, structure and composition, preparation methods, nucleic acid delivery and subcellular trafficking, biological activity, and mechanism of action. Linda Beckert, Alexander Philipp, and Ernst Wagner discuss receptor-targeted polyplexes in Chapter 13. The topics covered include polyplex design, plasmid DNA versus siRNA delivery, cellular targeting and intracellular delivery, bioresponsive systems, remaining challenges, and

clinical trials. Ampornphan Siriviriyanun and Toyoko Imae review dendrimers and dendrigrafts in Chapter 14. Some topics covered include dendrimer-DNA complexes, intracellular trafficking of complexes; dendrimer conjugates with PEG, cyclodextrins, or amino acids; gene therapeutics using dendrigrafts and their derivatives, and future directions. In Chapter 15, Shawna Shirley, Loree Heller, and Richard Heller provide a thorough review of gene electrotransfer. The topics covered include the history of electroporation, theories of electrotransfer, electrotransfer of nucleic acids and other agents, electrode selection and design, parameter selection, tissue-specific delivery, numerous therapeutic applications, safety, preclinical studies, and clinical trials. In Chapter 16, Christian Plank, Olga Mykhaylyk, and colleagues review magnetofection for enhanced and targeted delivery of nucleic acids. The topics covered include the principles of magnetofection, gene delivery vehicles and magnetic particles including their assembly and modifications, targeted delivery, magnets, commercial sources for magnetic reagents and magnets, in vitro and in vivo applications including use in humans, remaining challenges, and future directions. José Lanao, Carmen Gutiérrez Millán, and Clara Colino Gandarillas cover cell-based delivery systems in Chapter 17. The topics covered include preparation and therapeutic applications of carrier erythrocytes, production and applications of bacterial ghosts, stem cells for drug and gene delivery, neural stem cells, dendritic cells, mesenchymal stem cells, exosomes, microencapsulated stem cells, preclinical studies, and clinical applications. In Chapter 18, Zheng Fan and William Powers discuss pressurized transvenous-retrograde extremity perfusion (PREP). The topics covered include delivery to skeletal muscle, animal size versus biodistribution; PREP in dogs, nonhuman primates, and humans; preclinical studies to treat muscular dystrophy; optimization of pumps used in PREP; clinical trials; and results. Nancy Smyth Templeton provides an overview for the optimization of nonviral delivery in Chapter 19. The topics covered include optimizing delivery formulations, lipids and liposome morphologies, improving gene expression, encapsulation, flexibility, optimal colloidal suspensions, serum stability, circulation half-life, reversible masking, cell entry mechanisms, targeted delivery, biodistribution, barrier penetration in vivo, plasmid design, plasmid DNA preparation, administration routes, and clinical trials.

Section II covers other therapeutic strategies including technologies that knockdown gene expression. In Chapter 20, Frank Bennett, Eric Swayze, Scott Henry, and Richard Geary review antisense oligonucleotide–based therapeutics. The topics covered include antisense mechanisms of action, antisense oligonucleotide chemistry, oligonucleotide pharmacokinetics and toxicology, oligonucleotide formulations, clinical trials, FDA-approved antisense drugs, and future directions. Akin Akinc, Brian Bettencourt, and Martin Maier discuss RNA interference (RNAi) therapeutics in Chapter 21. This chapter covers the history to current applications of siRNA, mRNA knockdown mechanism, sequence selection, safety and off-target effects, immune stimulation, stability and potency, delivery including targeting, clinical trials, and future prospects. Chapter 22, written by David Soto-Pantoja, Jeffrey Isenberg, and David Roberts, provides an overview and current uses of morpholinos. The topics covered include in vitro and in vivo delivery, therapeutic applications (for treating cancer including solid tumors and inducing radioresistance, stress-driven hyper-prolactinemia, muscular dystrophies, hepatitis C, myotonic dystrophy, cardiovascular disease, ischemic injury, diabetes, West Nile and Ebola viral infection, Marburg hemorrhagic fever, Japanese and St. Louis encephalitis viral infection, influenza, viral myocarditis, bacteremia, and other diseases), toxicology, and clinical applications of morpholinos. Tina Catela Ivkovic and George Calin review microRNA therapeutics in Chapter 23. The topics covered include description and function of microRNAs (miRNA), miRNA biogenesis, mechanisms of action, miRNAs in human diseases, therapeutic approaches and novel strategies, clinical trials, and future directions.

Section III of the book covers gene expression, regulation, and detection. Sanjiv Sam Gambhir, John Ronald, and Sandip Biswal review imaging technologies in Chapter 24. The topics covered include instrumentation for imaging living subjects, reporter genes and probes, strategies for tracking delivery vehicles and transfected/transduced cells, noninvasive imaging of cell trafficking, monitoring gene therapy levels, clinical applications, and future directions. Nuria Vilaboa and Richard Voellmy provide a thorough review of deliberate regulation of transgenes in Chapter 25. The topics covered include tetracycline-responsive, streptograminsresponsive, phloretin-adjustable, L-arginine-regulated, uric acid-responsive, vanillic acid-responsive, ecdysone receptor–based, progesterone receptor–based, estrogen receptor–based, dimerizer-activated, radiation-induced, light-inducible, heat-inducible, ultrasound-inducible, *HSP70* promoter–based, and other gene switches; small molecule-dependent transactivators; transcriptional targeting; tissue-restricted/specific promoters; spatial and temporal regulation; chimeric transcription factors; dynamic range improvements and other strategies; preclinical studies; and clinical trials. Michele Calos and Lauren Woodard review nonviral genome modification strategies in Chapter 26. The topics covered include integrating versus nonintegrating gene therapy systems, transposon systems, phage integrase systems, nuclease systems and homologous recombination, comparison of all systems, and future directions. In Chapter 27, Jean-Pierre Gillet and colleagues review selectable markers for use in gene therapy. The topics covered include selectable markers, use of selectable markers to treat malignant hematopoietic system and nonmalignant diseases, episomal vectors for expressing selectable markers, vector engineering and optimization including bicistronic vector construction, selectable genes and markers for stem cell gene therapy, maintenance of long-term gene expression, clinical trials, and future directions.

Section IV covers gene and cell therapies, disease targets, clinical trials, and regulatory issues. In Chapter 28, Sadaf Amin and Shuibing Chen discuss pluripotent stem cells and

their therapeutic applications. The topics covered include definition of stem cells, self-renewal, potency, pluripotency, stem cell derivation, differentiation into various lineages, different stem cell types, replacement therapy, disease modeling and drug screening, therapeutic applications, and future directions. Amritha Kidiyoor, Anthony Atala, and Sean Murphy provide an extensive review of adult lung stem cells in Chapter 29. Some of the topics covered include the respiratory system, classical versus nonclassical stem cell hierarchy, assays for self-renewal, facultative stem cells, lung development during embryogenesis, cells involved in lung development and in steady state maintenance and injury, various lung injury models, adult airway epithelial progenitors in steady state maintenance and repair, region-specific progenitors, remaining challenges, and further work required for appropriate clinical applications. Ornella Parolini and colleagues provide a thorough review of placenta-derived cells and their therapeutic applications in Chapter 30. The topics covered include placenta structure and main cell types/tissues, stem/progenitor cells, isolation and preparation methods for the different cell types, various distinguishing cell markers, cell maintenance, induction, differentiation, studies in numerous preclinical models, and several clinical trials. Xuejun Parsons provides a detailed overview of human embryonic stem cells (hESCs) in Chapter 31. Topics include advantages of hESCs, proper establishment and maintenance of defined clinical-grade hESCs, histone and epigenome status of pluripotent hESCs, multicellular 3D models, direct conversion of pluripotent hESCs into cells for regenerating the central nervous system (CNS) and myocardium, and future prospectives.

Peggy Lange, Jonathan Fishman, Paolo De Coppi, and Martin Birchall review the surgical and translational aspects of tissue engineering in Chapter 32. The topics covered include an overview of tissue engineering and scaffolds and cells used in tissue engineering; clinical applications to skin, bone, bladder and urethra, cardiac tissues, airways, and other organs; preclinical studies; clinical trials; and future directions. In Chapter 33, Jian Yan and David Weiner review DNA vaccines. The topics covered include history and importance of vaccines; concepts and mechanism of DNA vaccines; strategies for vaccine improvement; vaccine delivery, molecular adjuvants; vaccines for infectious diseases, cancer, and autoimmune diseases; clinical trials; and future directions. Michael Katz, Anthony Fargnoli, Richard Williams, and Charles Bridges provide a thorough review of cell and gene therapies for the treatment of cardiovascular disease in Chapter 34. The topics covered include background of cardiovascular diseases including new statistical data for morbidity and mortality, numerous stem cells and other cell types used for cardiovascular cell therapy of the heart and vessels, cell-based regeneration and survival of transplanted cells, delivery routes and approaches for cell therapy, nonviral and viral vectors for cardiovascular gene therapy, uptake and intracellular trafficking of delivery vehicles, several different delivery routes for vectors, cardiovascular molecular targets, other gene and cell therapy approaches, and future directions. In Chapter 35, Elizabeth Kang and Harry Malech review gene therapy for the treatment of hematopoietic disorders. The topics covered include murine-based retroviral vectors, ex vivo gene transfer and transplantation of transduced hematopoietic stem cells (HSCs), ex vivo gene transfer and infusion of lymphocytes, HSC and lymphocyte gene transfer clinical trials, remaining challenges, and future directions. Uta Griesenbach and Eric Alton cover gene therapy preclinical studies and clinical trials for the treatment of cystic fibrosis in Chapter 36. The topics covered include the cystic fibrosis transmembrane conductance regulator gene, airway gene transfer agents, cystic fibrosis (CF), current standard treatments, therapeutic gene-based strategies, preclinical studies, clinical trials, the UK CF Gene Therapy Consortium, and remaining challenges. In Chapter 37, Deniz Dalkara and José-Alain Sahel review gene therapy for vision disorders. The topics covered include retinal diseases and their characteristics; gene therapeutic approaches for treating retinal diseases including gene augmentation/replacement, gene correction, interference strategies, survival factors, and optogenetics; vector requirements; routes of administration; immune privilege; clinical trials; and remaining challenges. Cell and gene therapies for the treatment of hearing disorders are covered in Chapter 38 by Lisa Gillespie and colleagues. The topics covered include review of conductive and sensorineural hearing loss; current standard treatments; delivery of therapeutic agents including drug-, gene-, and cell-based therapies; cell encapsulation/immunoisolation; repair and regeneration; stem cells for the replacement of hair cells and neurons; various target cells; different routes for delivery; preclinical studies; clinical trials; remarkable current advances; and remaining challenges.

In Chapter 39, Susan Samson, Vijay Yechoor, and Lawrence Chan review molecular therapies for the treatment of type 1 and type 2 diabetes. The topics covered include background for diabetes, gene therapy for hyperglycemia and metabolic derangements, transfer of glucose-responsive insulin transgenes, transfer of development/transcription factors to induce production of beta cells in the liver, modulation of immune derangement, gene therapy to combat insulin resistance, targets for peripheral and central control of obesity, other targets for glycemic and metabolic control, gene therapy for beta cell failure, preclinical studies, remaining challenges, and future directions. In Chapter 40, Norman Miller covers gene therapy for the treatment of familial lipoprotein lipase (LPL) deficiency and alipogene tiparvovec (Glybera®), the first gene therapy product approved for clinical use in Europe. This chapter covers the biochemistry and genetics of familial LPL deficiency, its clinical manifestations, the LPL gene and the AAV1 vector encoding the human LPL^{S447X} variant cDNA, preclinical development, and clinical trials including demonstrated efficacy. Sara Benedetti, Giulio Cossu, and Francesco Tedesco discuss the fields of gene and cell therapies for the treatment of numerous muscular dystrophies in Chapter 41. The topics covered include review of skeletal muscle and damage/degeneration, characterization and differences among the muscular dystrophies, the dystrophin gene, transcript and gene repair, gene replacement, allogeneic and ex vivo–corrected progenitor/stem cells, satellite

cells, myogenic progenitors, preclinical studies, clinical trials, and future directions. Louise Rodino-Klapac and Jerry Mendell review some gene therapy clinical trials for the treatment of muscular dystrophy in Chapter 42. The topics covered include Duchenne and limb girdle muscular dystrophies, disease progression, standard care, optimization of AAV vectors, preclinical studies, and clinical trials utilizing gene replacement, exon skipping, small molecules that bypass nonsense mutations, and vascular delivery approaches. Lisa Scherer and John Rossi review RNAi-based gene therapy for the treatment of HIV infections in Chapter 43. The topics covered include background of T-cells, stem cells, viral vectors, and HIV; multitargeting RNAi approaches to inhibit HIV replication; immunostimulation by RNAi; lentiviral vector triple HIV therapeutic preclinical studies; expanding the repertoire of HIV targets; and future directions. In Chapter 44, Carlos Ramos, Barbara Savoldo, and Gianpietro Dotti review numerous gene therapeutics and strategies for the treatment of cancer from the laboratory to the bedside. The topics covered include gene transfer strategies, tumor cell modifications, tumor environment modifications, antitumor response enhancement approaches, preclinical studies, clinical trials, and remaining challenges. Humberto Lara-Guerra and Jack Roth review gene therapy for the treatment of lung cancer in Chapter 45. This chapter covers non–small cell lung carcinoma, molecular profiling, gene replacement, tumor suppressor genes, tissue microarrays, gene therapy results in preclinical and metastatic cancer models, gene replacement in combination with chemotherapy, Gendicine (the first gene therapy agent approved for human use in China), clinical trials, and future directions. John Nemunaitis, Donald Rao, and Neil Senzer review cancer vaccines including a novel cancer vaccine called FANG in Chapter 46. Some of the topics covered include history of immune therapy in cancer, transforming growth factors beta, GMCSF, reversing immunosuppression, TGFβ2 antisense and GMCSF vaccine, Furin blockade to control $TGF\beta_1$ and $TGF\beta_2$, bifunctional RNAi targeting furin, and Phase I clinical trial results. In Chapter 47, Jean Christopher Chamcheu, Vaqar Adhami, Imtiaz Siddiqui, and Hasan Mukhtar review cutaneous cell and gene therapies for treating inherited and acquired skin disorders. The topics covered include the structure and function of human skin, delivery and gene transfer to the skin, ex vivo and in vivo gene therapeutic strategies, in vitro and animal disease models including a humanized murine skin model, disease targets, therapeutic approaches, protein replacement, cell-based therapeutics, revertant mosaicism therapy, preclinical studies, clinical trials, and future directions. Jonathan Karnes, Ying Zhang, and Ming Pei review cell therapies for cartilage production and clinical trials in Chapter 48. Topics covered include chondral and osteochondral injuries, subchondral bone, cartilage restoration, autologous chondrocyte implantation, stem cells for cartilage repair, mesenchymal stem cell–based cartilage repair, clinical trials and limitations, and future directions. In Chapter 49, María José Gómez-Lechón and colleagues provide a thorough and interesting overview of cell therapies for the treatment of inborn metabolic errors covering the history of liver transplants and hepatocyte transplantation through to current clinical trial results. This chapter also reviews optimized procedures for isolating and preserving hepatocytes for use in the clinic, hepatocyte transplantation and engraftment, additional types of stem cells for use in liver cell therapies, and clinical applications for these technologies. Rebecca Lim, Jean Tan, and Euan Wallace review cell therapies for the treatment of lung disease in Chapter 50. They cover common lung diseases in infants, children, and adults including standard treatments; lung stem and progenitor cells, mesenchymal stem cells, and other cell types; stem cell strategies for treatment; bioengineering new lungs; and clinical trials. In Chapter 51, Massimo Fiandaca and colleagues cover gene therapeutics for the treatment of neurological diseases (Parkinson's disease, Alzheimer's disease (AD), lysosomal storage diseases, Huntington's disease, stroke, epilepsy, motor neuron disease). The topics covered include the CNS, characterization and mechanisms of several neurological diseases, disease models, vector selection and delivery, promising gene therapeutics and approaches, clinical trials, and future goals. In Chapter 52, Alan Nagahara and Mark Tuszynski review gene therapy for the treatment of AD. Some topics covered are neurodegenerative disorders, standard treatments for AD, AD symptoms and neuropathology, AD gene therapy, nerve growth factor (NGF), NGF ex vivo and in vivo gene therapy and clinical trials, BDNF gene therapy, other gene therapy approaches, and future directions. Chapter 53 provides an up-to-date perspective on the use of stem cell therapy to treat AD, written by Cesar Borlongan and colleagues. This chapter also presents an overview of AD, successful and unsuccessful approaches for treatment, neural stem cells for the treatment of various neurological diseases, proper stem cell purification, and challenges in the successful use of stem cells to treat AD in the clinic. Finally, in Chapter 54 Daniel Takefman and Lilia Bi, from the Food and Drug Administration (FDA), extensively cover the current regulation of gene therapy in the United States. The topics covered include oversight, the IND process, pre-IND meetings, preclinical development, Master File submission, CMC review, related agencies such as the Recombinant DNA Advisory Committee (RAC), review considerations for early- and late-phase studies, specific safety issues, Good Manufacturing Practices (GMP), guidance documents, and other helpful references and Internet links.

Once again, the authors have done an outstanding job providing readers with broad knowledge, insight, and tools available in the constantly evolving fields of gene therapy, cell therapy, and tissue engineering. The ongoing development of these therapies draws from many disciplines including cell biology, virology, molecular biology, medicine, genetics, immunology, biochemistry, physiology, chemistry, bioengineering, biophysics, molecular imaging, microbiology, pharmacology, and toxicology. This book includes

topics in these areas and has been planned to facilitate its ongoing use as a textbook for classes in gene and cell therapies, gene transfer, and therapeutic applications. The book is also suitable for readers from all backgrounds including students, physicians, scientists, and other individuals interested in understanding the current status of gene and cell therapies and their contribution to the field of medicine. Several contributors have broadened their introductions to facilitate use of this edition as an educational textbook and have also contributed information to provide the context for their focused research efforts and for current technologies, disease targets, and clinical applications.

Editor

Nancy Smyth Templeton earned her PhD in molecular biology and biochemistry from Wesleyan University, Middletown, Connecticut. She underwent postdoctoral training at the National Institutes of Health in the NCI and the NHLBI, Bethesda, Maryland. She was recently the director of Delivery Systems at Gradalis/Strike Bio Inc., Carrollton, Texas, that focuses on targeted delivery of bifunctional shRNA therapeutics to solid tumors by intravenous injections using robust nanoparticles, bilamellar invaginated liposomes (BIVs). These BIVs have already demonstrated efficacy for the treatment of many cancer types in animal models and in clinical trials. Dr. Templeton has served on numerous review panels, study sections, professional committees; is an ad hoc reviewer for several peer-reviewed journals; is editor of the three prior editions of this book; and is a member of many professional societies. She has 62 peer-reviewed publications, presented over 170 seminars as an invited speaker and/or moderator including national and international meetings, has been a consultant for five biotech and pharmaceutical companies, has 16 issued patents and 6 additional patent applications filed for recent technology developed, has contributed to five different clinical trials, has served as the principal investigator of a laboratory and faculty member at the Baylor College of Medicine (BCM), Houston, Texas. Dr. Templeton has performed extensive funded research from the NIH and other agencies, and has won several awards including two distinct Fulbright & Jaworski LLP Faculty Excellence Awards for teaching accomplishments at BCM.

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