

# Translating Gene Therapy to the Clinic

Techniques and Approaches

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*Edited by*

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# Preface

In the summer of 1968 two staff scientists at Oak Ridge National Laboratory, Stanfield Rogers and Peter Pfuderer, suggested in a letter to *Nature* that “viral RNA or DNA information” could be used in the control of genetic deficiency diseases as well as nonheritable disorders such as cancer.<sup>1</sup> Their proposition was based, like many scientific breakthroughs, on an experiment of nature: the observation that circulating arginine levels are elevated in humans following infection with Shope papilloma virus, which was thought to induce a virus-specific arginase. Theirs was a prescient thought, borne on the eve of the birth of recombinant DNA technology. But it took some four decades to begin realizing its promise.

The first approved use of gene therapy occurred in 1991 under the direction of W. French Anderson. Ashanti DeSilva was a four year old girl with the enzyme-based immune deficiency disorder ADA-SCID. Retrovirus-mediated transfer of an adenosine deaminase gene into her autologous T cells led to a clinical response, albeit incomplete and temporary.<sup>2</sup> This was followed by a “loss of innocence” attendant on the treatment, in 1999, of Jesse Gelsinger, an 18 year old man with ornithine transcarbamylase deficiency.<sup>3</sup> He died as a consequence of an adenovirus vector-associated inflammatory process. Shortly thereafter five infants with SCID-X1 developed acute leukemia after receiving a murine retrovirus-based gene therapy to replace a defective interleukin 2 receptor H chain.<sup>3</sup> But then only one of those five patients died from their leukemia. And with a final enrollment of 20 SCID-X1 infants, and correction of severe immune deficiency in 17 of them over a median follow up of 9 years, gene therapy was finally established as a realistic therapeutic for those without alternatives.<sup>4</sup>

Since that time over 1800 gene therapy trials in 31 countries have been initiated or completed.<sup>4</sup> And the field’s promise is not restricted to “simple” replacement or excision of a defective gene. For example, genetic engineering techniques have been used to inculcate tumor recognition or virus resistance in autologous lymphocytes of patients with metastatic cancer<sup>5</sup> and advanced AIDS.<sup>6</sup> Although there are currently no U.S. FDA licensed gene therapy products, in 2012 Glybera (alipogene tiparvovec) became the first example of this technology to be approved for clinical use, in Europe, after its endorsement by the European Medicines Agency.<sup>7</sup> Based on an adeno-associated virus type 2 (AAV2) vector, Glybera corrected a defect in the lipoprotein lipase gene that otherwise leads to severe pancreatitis. Like most new technologies Glybera is expensive—about \$1.6 million per patient—partially related to the ultra-orphan nature of the target disorder. (There are only a few hundred cases annually in the resource rich world.<sup>7,8</sup>) But its clinical success, as well as preliminary data from phase 1/2 and phase 3 clinical trials for more common conditions, as outlined in our text, has led to an explosion in commercial interest; between 2013 and early 2014 US companies have invested some \$600 million in gene therapy research.<sup>9</sup>

The text you are about to explore is an introduction for the translational and basic researcher as well as the clinician to the vast field of gene therapy technology. It is the first book in a new series, *Advances in Translational Medicine*. The project is a direct outgrowth of our editing of an illustrious journal, *Translational Research, The Journal of Laboratory and Clinical Medicine*. It is coincident with the journal’s celebration of a legacy of 100 years in the promotion of excellence in clinical and translational research. This first volume is also a perfect opportunity to congratulate the Central Society for Clinical and Translational Research (CSCTR), a key partner with the journal. Albeit technically only in its 87th year, CSCTR traces its heritage to the Central Interurban Clinical Club, the establishment of which, in 1919, places it not far off the 100-year mark. Its fundamental goals, shared by our journal and this series, are critical and constant. Above all, champion the young investigator, bring in new ideas, establish diverse collaborations, and limit inbreeding. The special topics issues published annually in *Translational Research* are highly quoted. They achieved sufficient notice that the book division of Elsevier, publisher of our journal, began this series based upon expanded versions of our special issues and invited reviews.

Early on, the national importance of our society was well recognized. It also had an unanticipated impact on gene therapy related issues. The policies of genetic modification in clinical trials are regulated by the Declaration of Helsinki. And in 1966, only four societies were requested to endorse this declaration relating to “ethical principles for medical research involving human subjects”; the American Medical Association, the American Society for Clinical Investigation, and the American Federation for Clinical Research joined us. This declaration, along with the 2001 HUGO (Human Genome

Organization) consensus, covers the types of somatic gene therapies discussed in our text. Germline gene approaches by which gametes (sperm or ova) are modified, permitting a therapeutic manipulation to be passed on to future generations, are proscribed for ethical reasons in many countries, and are not covered here.

The authors of the following chapters are leaders in the field of gene therapy. They cover a range of topics and technologies with a depth and clarity to be commended, providing helpful illustrations and comprehensive citations to the literature. Several chapters focus on specific diseases, while others cover new technologies or barriers to progress. It strives to cover, in depth, disease-specific areas of particular promise. Its initial focus is on mechanisms of introducing a gene, generally via a viral vector, that either: (1) causes a protein to be expressed in a patient with a defective protein product, or two little of the normal one; or (2) introduces editing genes, “molecular scissors” that excise or disrupt genes causing a disease. As the field has evolved to encompass non-DNA-based technologies, utilizing antisense oligonucleotides, small interfering RNAs, etc. that do not alter a gene, but directly interact with RNAs or proteins, are also presented here.

These chapters also provide roadmaps to the ontogeny of current gene therapy trials and methods by which a group might design their own. I have borrowed a recently published patient-centered approach to designing a gene therapy for epilepsy<sup>10</sup> as an example of how the introductory chapters of this text set the stage for strategies to tackle your own areas of therapeutic interest.

1. Choose an animal model that accurately reflects the clinical problem in which to conduct preclinical studies.
2. Decide on a therapeutic approach. This is simpler when a single-gene defect is involved, limiting a functional protein product correctable by a relatively small increase in that product, as in hemophilia B. In a complex phenomenon such as epilepsy, one needs to decide if the target might best be decreasing neuronal excitation or increasing neuronal inhibitory pathways. Targeting of an entire cohort of genes could be contemplated.<sup>10</sup>
3. Choose a safe, effective vector. At the moment this usually means AAV, in which case limited payload size is a major impediment, or a lentivirus. But retrovirus, adenovirus, herpes simplex virus, plasmid, and other transport systems are also in various stages of clinical testing, and are outlined herein.
4. Consider all potential obstacles and explore them. Our text considers issues of payload toxicity, vector targeting, sufficiency of gene product expression, and the limits of in vitro and animal models. It also touches upon potential regulatory issues and good manufacturing-practice costs, but related details are left to other sources. For example, the American Society of Gene & Cell Therapy offers Web sites with information on issues related to the conduct of clinical gene therapy trials and the regulatory issues they raise.

This book provides coverage of the full spectrum of scientific and clinical progress, emphasizing new approaches in the field that currently have the greatest therapeutic application or potential and those areas most in need of future research. Serving both as an introduction to the field of gene therapy and as a general reference, it should prove an invaluable resource for both the expert and new investigator entering the field, as well as the clinician considering enrolling patients in clinical trials.

**Jeffrey Laurence, M.D.**  
**August 2014**

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# About the Editors



Dr. Jeffrey Laurence is Professor of Medicine at Weill Cornell Medical College, Attending Physician at New York Presbyterian Hospital, and Director of the Laboratory for AIDS Virus Research at those institutions. He is also Senior Scientist for Programs at amfAR, The Foundation for AIDS Research, co-founded by Dr. Mathilde Krim and Dame Elizabeth Taylor, and Editor-in-Chief of AIDS Patient Care and STDs and Translational Research (formerly the Journal of Laboratory and Clinical Medicine).

Dr. Laurence received his B.A. Phi Beta Kappa, summa cum laude from Columbia University in 1972, and his M.D. with honors from the University of Chicago Pritzker School of Medicine in 1976. He was elected a Rhodes Scholar to Oxford University in 1973. Deferring this honor, he accepted a Henry Luce Fellowship to Japan, where he worked at the Institute for Cancer Research in Osaka from 1974–1975. Dr. Laurence returned to New York to complete a residency in internal medicine and fellowship in hematology-oncology at The New York Hospital, followed by a research fellowship in immunology at The Rockefeller University.

His work focuses on the mechanisms by which HIV and antiretroviral drugs used in its treatment affect endothelial cells and osteoclasts, in models for thrombosis, cardiovascular disease and osteoporosis linked to HIV. As an outgrowth of this research he is has a long-standing interest in exploring thrombotic microangiopathies associated with complement activating disorders.

Dr. Laurence is a member of several national and international AIDS organizations, and recently received an “Award of Vision” from the Red Ribbon AIDS Foundation. He is also a recipient of the Clinician-Scientist Award of the American Heart Association and the William S. Paley Fellowship in Academic Medicine, and is an elected Fellow of the New York Academy of Sciences and a member of the American Society for Clinical Investigation. He has 3 children and lives in Greenwich, CT.



Michael Franklin, MS is a medical editor in the Division of Hematology, Oncology, and Transplantation (HOT) at the University of Minnesota. He earned his M.S. in science journalism with honors from Boston University in 2000. While in Boston, Mr. Franklin interned at the *Harvard Health Letter* at Harvard Medical School and *Boston Review* at Massachusetts Institute of Technology. He was also a staff writer for *The Daily Free Press*, the Boston University school newspaper, and contributing editor for *Stellwagen Soundings*, a newsletter covering the Stellwagen Bank National Marine Sanctuary. Shortly after graduation, Mr. Franklin began his editorial career as the Managing Editor of *The Journal of Laboratory and Clinical Medicine*, subsequently retitled *Translational Research* in 2006. During his tenure as Managing Editor, Mr. Franklin developed an interest in publication ethics while mediating breaches of scientific misconduct involving authors of the journal. He has written about publication ethics for *Minnesota Medicine* and teaches a seminar on the topic to HOT trainees. Michael has a long-standing interest in the history of science, specifically the history of experimental discoveries in chemistry and medicine, and how scientific reasoning works as an engine of human knowledge. Since becoming a medical editor in 2006, Michael has written or edited hundreds of research reports, grant proposals, book chapters, reviews, educational curricula, and other science-related material for clinicians and scientists. He regularly teaches seminars on writing in the sciences, designing visual displays of data, and how to read a journal article to HOT faculty and/or trainees, as well as other groups, including the *Association of Multicultural Scientists* and the *North Central Chapter* of the *American Medical Writers Association* (AMWA). He served as President of the *North Central Chapter* from 2010–2011 and has been a member of AMWA since 2007. When not writing or editing, Michael spends time with his partner, two daughters, and dog in Minnetonka, Minnesota.

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## Chapter 1

# Translating Genome Engineering to Survival

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*“Daring ideas are like chessmen moved forward; they may be beaten, but they may start a winning game.”*

Goethe

## LIST OF ABBREVIATIONS

**DNA** Deoxyribonucleic acid

**HCT** Hematopoietic cell transplantation

**iPSCs** Induced pluripotent stem cells

**mRNA** Messenger ribonucleic acid

**SCID** X-linked severe combined immunodeficiency

**CRISPR** Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system

## 1.1 ORIGINS

The process of research and its clinical translation is not just driven by doctors and scientists; they are driven by the patients themselves. In the boundaries between science and society, there is a subset of individuals with genetic disorders for whom simply living life is inherently unsafe. These patients and their families frequently view risks of therapy very differently than healthy people do. Probabilities of success or failure that would be unthinkable for a healthy person can be acceptable to those with debilitating or fatal diseases. These extreme situations, in which the possibility of benefit outweighs the significant risk, are fortunately rare but critical to clinical translation. Human suffering is the same whether it is caused by a rare or a widespread disease, but the attention of experts and commitment of society may differ between the two conditions. Because of this disparity, it is important to stress that gene therapy approaches will apply equally to the orphan diseases in which they are pioneered and to the diseases and injuries that cause much of the morbidity and mortality in the world.

Gene therapy has been on the horizon for over 30 years. Encouraged by its early promise, patients and their despairing families have had their hopes raised again and again only to be repeatedly disappointed. However, in 2014, evidence of clear success in selected diseases and the application of safer technologies have added to the intellectual mass needed to forward the process of bringing gene therapy to the clinic, thus rendering countless grim disorders treatable and ultimately curable.

The concept of gene therapy for genetic disorders has its origins in Gregor Mendel's theory that cells contain small units that are messengers of inherited characteristics<sup>1</sup> and Erwin Schrödinger's insight that genes are blueprints of cellular function.<sup>2</sup> Gene therapy is one of the most appealing theories in biomedicine because it is aimed at the cause rather than the symptoms of the disease. However, the ability to harness the potential of gene transfer or DNA repair to correct a specific genomic lesion has simultaneously been one of the most daunting concepts to put into practice, although such “corrective” mutations can occur spontaneously in the setting of somatic cell mosaicism.

Mosaicism, the naturally occurring, spontaneous restoration of function in revertant cells, has been reported in patients with several genetic diseases: the skin disease epidermolysis bullosa; the metabolic disorder hereditary tyrosinemia

type I; the bone marrow failure syndromes Fanconi anemia and dyskeratosis congenita; and the primary immunodeficiencies, Wiskott–Aldrich syndrome, Bloom syndrome, X-linked severe combined immunodeficiency (SCID), and adenosine deaminase (ADA)-deficient SCID.<sup>3–14</sup> Therefore, in theory any genetic disease can be spontaneously corrected by a gene conversion, compensatory mutation in *cis*, or by intragenic crossover between maternal and paternal alleles with two different mutations in the same gene.<sup>15</sup>

Despite this knowledge, there clinically are several blocks to applying revertant mosaicism. For example, it remains unclear why in some conditions the self-correction occurs rarely and why in others it may occur repeatedly even in a single individual.<sup>16–18</sup> Furthermore, when self-correction does occur, it may not be clinically meaningful because the revertant event is unpredictable in the molecular mechanism of reversion (and may restore the gene function only partially) and in the location in the gene. Also relevant is the unpredictability of the type of cell in which the reversion occurs, ranging from cells and tissues relevant to the disease phenotype to those that are functionally neutral. It is also currently impossible to predict the type of cell where revertant mosaicism will occur: in stem cells with significant repopulation potential, in progenitor cells—more differentiated but still possessing a variable degree of “stemness”—or in fully differentiated postmitotic cells with limited functional effect beyond the corrected cell.

If revertant mosaicism does not supply the clinical answers we seek, then where do we look next? To see the future, it is useful to first examine the past.

### 1.2 SYNCHRONICITY OF DISCOVERIES

The foundations for the practice of stem cell gene therapy today and for optimism about its future effect are three unrelated discoveries made 60 years ago in the 1950s. They are the following:

1. Defining the structure of DNA.<sup>19</sup>
2. Inducing immunological tolerance by tissue transplantation.<sup>20</sup>
3. Creating induced pluripotency by removing the nucleus of an egg and putting in the nucleus of a differentiated cell.<sup>21</sup>

Together, these three discoveries established the science needed for gene therapy to become a reality: from understanding the arrangement of the molecules in DNA, which allowed them to be rearranged; to understanding the mechanisms of immune tolerance, which allowed for cells and tissue to be transplanted; to being able to create a pluripotent cell from a gene-corrected one, which allows these cells to be multiplied and differentiated into different lineages to work in physiologically meaningful ways throughout the body.

### 1.3 GENE ADDITION

The discovery of the double-helix structure of DNA by James Watson and Francis Crick is obviously important to gene therapy because it showed that the nucleotides are organized in specific pairs. The understanding of this structure, and of the mechanism by which it divided, opened the new field of genome engineering, in which genes could be cut out of the genome of one cell and spliced into the genome of another as described in this volume by Gersbach. Gene therapy without this recombinant DNA technology is unimaginable.

#### 1.3.1 Genes as Medicine

Of the more than 1800 genetic disorders described in humans, only a small fraction can be treated and even fewer cured. At this time, we are seeing possible cures using gene therapy for sickle cell disease, thalassemia, and ADA deficiency. In a field with so many challenges, these imminent steps into clinical application remind us how important and beneficial successful methods of gene therapy will be. Even with this powerful motivation, there are formidable technological barriers to accomplishing successful gene transfer, such as crossing the cellular membrane, escaping from the endosome, moving through the nuclear membrane, and integrating into the host genome.

The first technological problem is crossing the cellular membrane, the divider between the cell and its environment, a protective barrier that is quite selective about what it will let into the cell. Large molecules such as DNA, which might compromise the cell's existing DNA, are kept outside. Should the DNA cross the membrane, it must then encounter another cellular defense, the endosome, where material from outside the cell is either broken down into harmless components to be expelled or encapsulated and sent forward for use in the cell. Should the first two obstacles be surmounted, the third and most difficult remains: how to get the cell's genome, neatly and strictly paired and sequenced, to break open and accept foreign DNA.

### 1.3.2 Viral Gene Therapy and the “Selfish” Transgene<sup>22</sup>

Many of these biological hurdles are surmounted by adapting viral vectors, which are already evolutionarily perfected to penetrate a cell and transfer DNA, for use in gene therapy.<sup>23</sup> This method has its own risks: reactivation of the original virus, formation of tumors or other cancers, and immune reaction to the viral components. The issues of immunity are discussed by Mingozi in another chapter in this book.

In terms of delivering a wild-type gene into a genome with a pathogenic mutation in the same gene, gene therapy as we know it today has been most advanced by its remarkably successful application to the therapy of primary immune deficiency states, namely SCID (X-linked and ADA) and Wiscott–Aldrich syndrome. Another triumph of gene therapy has been improvement of vision in individuals with Leber congenital amaurosis.<sup>24–27</sup> In this volume, McClements and MacLaren review gene transfer approaches to retinal disease with special consideration of the viral serotype and regulation elements of the therapeutic transgene.

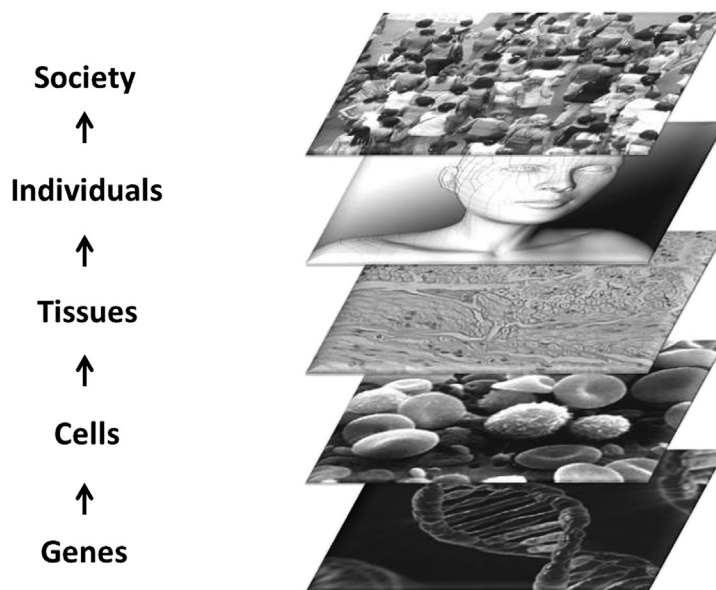
In addition to the many advantages, significant questions remain about the use of viral vectors. In the brief course of this century, close to 100 individuals with (mostly) fatal genetic disorders have been treated using autologous transplantation with gene-corrected cells that were transduced with retroviral vectors carrying the therapeutic gene. This strategy relied on delivering a functional gene along with exogenous regulatory elements that promoted sustained, high-level gene expression. However, the consequences of integrating the retroviral vector and its “selfish transgene” into the host genome included loss of physiological regulation of the treated gene and the disruption and possible dysregulation of other endogenous genes.<sup>28–30</sup> For example, in the French-British SCID trial, 5 of 20 individuals experienced integration of the viral vector and cargo in close proximity to an oncogene, activation of which led to clonal expansion and leukemia. Four of the five children with leukemia were successfully treated, but one died. Despite these serious complications, the overall outcome of the trial provided evidence that gene therapy is superior to the previous standard of care (i.e., bone marrow transplantation) by providing better immune function with polyclonal T-cell repertoire, increased disease-free survival (>80%), and a better quality of life.

### 1.3.3 Safety and Bioinformatics

The biological challenges to gene therapy have not been the only obstacles. In 1974, approximately a century after Mendel was so brilliantly right about genes as “atoms of heredity,” worries about the dangers of recombinant DNA technology led research scientists to create a voluntary moratorium of all research involving genome engineering.<sup>31</sup> In response, the 1975 Asilomar Conference on Recombinant DNA in Pacific Grove, CA, developed and released safety recommendations under which research could proceed.<sup>32</sup> This attempt by scientists to address public concerns through self-regulation focused attention on the research and resulted in nearly worldwide governmental regulation. In retrospect, the constraints of these recommendations may have delayed research efforts by focusing on “dangers” that, as one of the conference’s intellectual leaders commented decades later, “...were strictly conjectural (that is, you *thought* they were dangers).”<sup>33</sup> This emphasis on safety resulted in enormous efforts being redirected to the evaluation of off-target effects using comprehensive large-scale integration site analysis to identify common integration sites and to associate clonal dominance with malignant lymphoid or myeloid proliferation.

One of the advantages of gene transfer is its spectacular modularity: with various building blocks of the vector-cargo complex adapted to deliver to specific organ locations, to express in a tissue-specific manner, or to transduce cell types committed to cell-specific differentiation programs. Gene therapy can be designed for injuries, for systemic disorders, or for diseases limited to individual organs. In this book, Tang and Hammond provide an update on gene therapy for congestive heart disease. Frazier and Kontos cover the exciting possibilities of ex vivo gene modification of a venous graft used in revascularization bypass surgery for ischemic cardiovascular disease. Prickett and Jain summarize advances in gene transfer and gene repair of the cystic fibrosis transmembrane regulator gene, and a new perspective on the gene therapy of rheumatoid arthritis and osteoarthritis is given by Evans, Ghivizzani, and Robbins.

Looking forward, this information will be amplified by the application of bioinformatics, in which huge amounts of information can be analyzed for minute connections and more complex relationships revealed (see [Figure 1.1](#)). The current knowledge of common integration sites in clonally expanded hematopoietic cells may evolve into a “genome instability index” that takes into consideration the alteration of signal cascade maps, gene regulatory pathways, and the cellular state after transduction or transfection. Thus, this future directory would better map the neighborhoods of influence on the specific transgene and quantify the risks of gene transfer in a systematic, comprehensive way. The collective intelligence on genotoxicity has already made real the concept of large-scale, computer-automated amplification of knowledge<sup>34</sup> and will further advance with this kind of integrative model.<sup>35</sup> We are now in the early stages of computational science, which is based on modeling tremendously complex interactions among genes, cells, tissues, individuals, and societies (epidemiology), and moving steadily into international cooperation.



**FIGURE 1.1 Genes as medicine.** When we look at gene therapy from the viewpoint of a gene itself, we may not grasp the gene in the context of a cell, a tissue, or an organism that together give the gene its physiological meaning. Bioinformatics models the complex interactions among genes, cells, tissues, individuals, and society to help pinpoint the most productive areas of research. Although experiments and clinical encounters are typically confined to one method of observation, biological systems are complex, with several interconnected organizational and functional layers. A systems approach prevents treating experimental and clinical data in a fractured and intermittent way and offers a path from mechanistic understanding of disease to a rational algorithm of effective treatment of genetic disorders.

### 1.3.4 Nonviral Gene Therapy

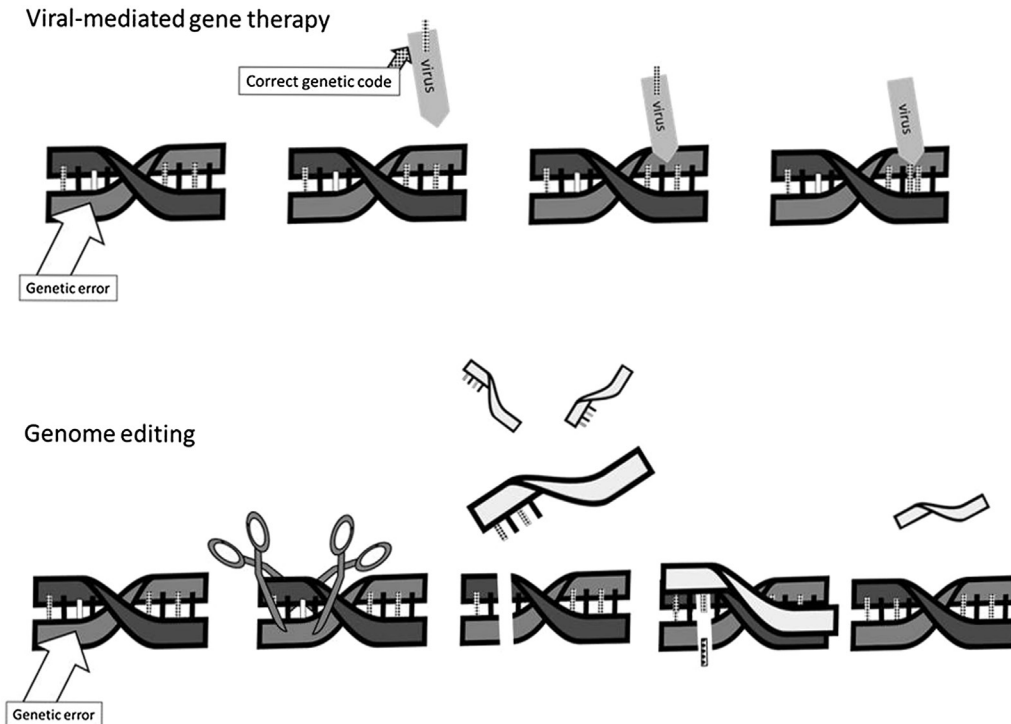
The expense associated with clinical-grade, viral-based gene therapy trials is rarely given much attention in academia, but it remains a major consideration for those who would conduct clinical trials. DNA-transposable elements are an elegant answer for the financial concerns, but their position effect after genome integration needs to be assessed with the same diligence with which viral vectors were evaluated. Hackett, Starr, and Cooper herein present a cautious, balanced view of insertional mutagenesis of transposons as a tool of discovery and as a challenge for clinical gene therapy.

## 1.4 FROM GENE ADDITION TO GENE EDITING

In efforts to improve the safety and efficacy of potential clinical therapies, the center of gravity for gene therapy may be shifting from gene addition (in which a whole new gene is pasted into the genome with the use of viruses or transposons) to genome editing (see [Figure 1.2](#)) (where the pathogenic mutation is corrected in its natural gene location with zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), *clustered* regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, or homing endonucleases).<sup>36,37</sup> Hybrid molecules are now being engineered to target a specific location in the genome and to introduce a break in the DNA proximal to the targeted mutation. The cleavage in the DNA is then resolved by homologous recombination between the endogenous genes and an exogenously introduced donor fragment containing the normal sequence. In this way, the pathogenic mutation is permanently changed back to the normal sequence. This has the added benefit of preserving the architecture of the genome and maintaining gene control under the normal cellular regulatory elements. Although further studies are needed, recent research gives evidence that genome editing may be highly effective and safe.<sup>38–41</sup>

## 1.5 THERAPY FOR GENETIC DISORDERS

Critical to allowing the transfer of material from one individual to another was a discovery from Peter Medawar's investigation of skin grafting. He found that tissue from one individual will be rejected by another individual because of an immune response to the donor transplant. Medawar, Billingham, and Baruch Brent<sup>20</sup> further showed how to manipulate the immune system to develop acquired immune tolerance. Their experiments, visually represented by a white mouse sporting a healthy spot of fur from a black mouse, demonstrated that specificity of immune response develops before birth and that a subject injected with a donor's cells in the prenatal period can later accept tissue from that donor's body. From a physiological standpoint, because



**FIGURE 1.2 Gene therapy.** Viral-mediated gene transfer results in the transfer of the correct genetic material, but usually in an almost random location. This added transgene may disrupt functions of other genes, potentially even causing cancer. By contrast, genome editing uses a donor template to correct the specific mutation in the genome and typically leaves nothing else behind. Thus, not only is the gene correction precisely located after gene editing but also nothing remains to disrupt normal genome function.

genes exist only in the context of a cell, the donor's cell only makes clinical sense when transplanted. Thus, immune tolerance of allogeneic wild-type or autologous gene-corrected grafts is the key to successful cell gene therapy.

### 1.5.1 Blood and Marrow Transplantation for Genetic Disease

Perhaps the first applied gene therapy (in a broad sense, i.e., delivery of a wild-type gene into a patient with a loss-of-function mutation in that gene) was accomplished by means of bone marrow transplantation. Pioneering work by Medawar, E. Donnell Thomas, and others<sup>20,42–45</sup> made it clear that transfer of allogeneic bone marrow cells can regenerate the lymphohematopoietic system. Equally impressive was the way their advances in allogeneic transplantation for malignant disease were taken in an entirely new clinical direction by Elizabeth Neufeld's discovery of enzyme cross-correction between cells derived from individuals with mucopolysaccharidosis type I (Hurler syndrome) and mucopolysaccharidosis type II (Hunter syndrome).<sup>46</sup> By pure coincidence, her findings were published the same month, November 1968, that Robert Good and his team performed the first successful in-human bone marrow transplant.<sup>47,48</sup> With this step, a new field of medicine originated—hematopoietic cell transplantation (HCT).

The concept of allogeneic transplantation as a therapy for genetic disorders has been applied to several conditions:

- Selected **enzymopathies** (e.g., mucopolysaccharidosis type I or  $\alpha$ -mannosidosis),
- **Bone marrow failure syndromes** (e.g., osteopetrosis, Fanconi anemia, and dyskeratosis congenita),
- **Neuronopathic disorders** (e.g., adrenoleukodystrophy and metachromatic leukodystrophy),
- **Hemoglobinopathies** (e.g., sickle cell anemia and thalassemia, and those discussed by Rivella),
- **Extracellular matrix disorders** (e.g., the genodermatosis epidermolysis bullosa), and
- **Severe immune deficiencies** (e.g., X-linked SCID, ADA-deficient SCID, Wiskott–Aldrich syndrome, and chronic granulomatous disease).<sup>49–58</sup>

Although a life-saving measure for patients with some diseases, allogeneic HCT can result in life-threatening side effects and must be seen and understood as a radical treatment used only for otherwise deadly disorders. These complications can occur either as a result of the physical injury associated with the chemotherapy and radiation used in the preparative regimen or from

the immune injury caused by the allogeneic nature of the hematopoietic graft. Injuries from the preparative regimen can present as systemic endothelial injury (sinusoid obstruction syndrome or veno-occlusive disease), renal toxicity, or pulmonary toxicity. Post-transplant immune injury leads to profound immunosuppression and, in a significant minority of HCT recipients, to an attack by the donor immune cells on the tissues of the recipient (termed graft-vs-host disease). Each of these serious and potentially fatal complications could be diminished or avoided by using the host's own cells (i.e., gene-corrected, autologous HCT).

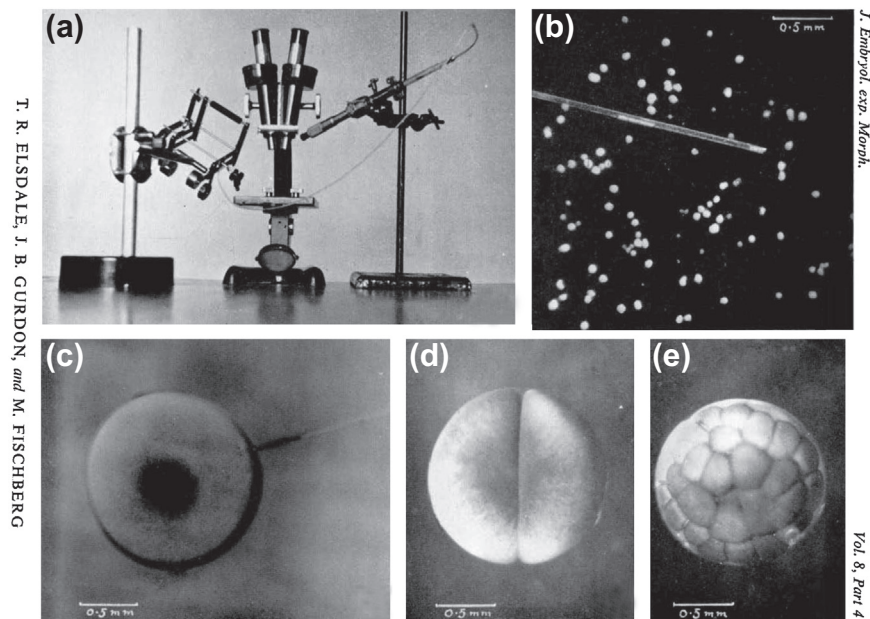
The use of viral and adenoviral vectors, as noted in *The Selfish Transgene*, has potential complications, such as random insertional mutagenesis and triggering oncogenic processes. Another method of gene therapy involves editing the genome in a “cut and paste” manner. Various technologies (e.g., zinc finger nucleases, TALENs and CRISPRs) are all showing potential in animal models. Additional considerations include choosing the appropriate cellular vectors and creating a clinically significant number of gene-edited cells for transplant.

### 1.5.2 Cellular Vectors

Clearly, for the any meaningful gene therapy strategy to work, it must target the cell type relevant to the specific disease. Although tissue-specific cell types such as keratinocytes and retinal epithelial cells have been successfully gene corrected in most cases, the effects of gene correction are amplified in proportion to the proliferative, repopulating capacity of the cellular vector. For this reason, stem cells have been the target cells of choice for several gene therapy applications. The most prominent example is using hematopoietic stem cell grafts in gene therapy trials for blood disorders.<sup>59–61</sup> In this volume, two chapters cover the evolving knowledge of the gene therapy of nonmalignant blood disorders. Dong, Rivera, and Breda describe lentiviral gene therapy trials for thalassemia and sickle cell disease, and Walsh and Batt focus on adenoviral gene transfer and spliceosome-mediated pre-mRNA trans-splicing in hemophilia. Gene therapy for neurological disorders has not, until recently, been seen as a promising application. Simonato, Wahlberg, Goins, and Glorioso evaluate current viral gene therapies and their potential in treating pain, epilepsy, and Parkinson's disease. Bertoni outlines current gene therapy approaches to Duchenne muscular dystrophy.

### 1.5.3 Induced Pluripotent Stem Cells

The ability to take one kind of cell, edit the genome, and then produce another type of cell that is more suitable for therapy was made possible by the work of Sir John Gurdon.<sup>21,62</sup> His work, based on pioneering work by Robert Briggs and Thomas



**FIGURE 1.3 Simple equipment for a complex procedure.** Photographs of the laboratory equipment used to successfully perform transfer of a nucleus from a mature intestinal cell into the egg cell of a frog, replacing the immature cell nucleus. The egg cell then developed into a viable tadpole, demonstrating that the DNA in all cells in the body contained the information needed to create the entire frog. (a) Low-power binocular microscope with Singer micromanipulator/microdissector. (b) Cells from donor embryo with micropipette. (c) Introduction of mature donor cell into frog egg. (d-e) Normal division of egg with new DNA.<sup>64</sup>

King,<sup>63</sup> established that DNA in a mature cell's nucleus contains the complete blueprint to reproduce the organism from which the cell came. This was tested by removing the nuclei from a mature cell and an immature cell. The nucleus from the mature cell was then inserted into the immature cell, and from this a complete organism (a frog) was then produced. The fantastic precision of this nuclear transfer between cells was performed using the most basic of equipment (see [Figure 1.3](#)).<sup>64</sup>

This basic science research later led to what is probably the most important significant development of the past decade relevant to stem cell gene therapy—the generation of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka. He was able to take a mature cell committed to specific tissue function and turn back its biological clock, returning it to a pluripotent state. From this pluripotent state, the cell could be selectively differentiated into other cell types, an essential element in the design of an appropriate cellular vector.

### 1.5.4 Stem Cell Gene Therapy

Most stem cell therapies to date have used organ-specific stem cells for organ-specific diseases, such as hematopoietic stem cells for blood disorders or epidermal stem cells for diseases of skin. Nevertheless, since the foundational discoveries of nuclear and cell reprogramming by Briggs, King, Gurdon, Yamanaka, and Thomson, the culture of embryonic stem cells, and the bioengineering of iPSCs<sup>21,62,63,65–71</sup>, it has been possible to think that these cell types may be useful as cellular targets for future stem cell gene therapy approaches and in regenerative medicine for disease and injury.<sup>72</sup> In this book, Mason and Kaufman discuss the history of iPSCs and the manipulation of iPSC genomes. This evaluation of efficacy and safety in the clinical translation of cells derived from gene-corrected iPSCs is especially important because the risk and benefits of stem cell therapy are balanced with those of gene therapy.

Disease-specific challenges will remain. For example, in DNA repair-deficient Fanconi anemia cells, the bone marrow cells for gene correction are few in number, extraordinarily intolerant to *ex vivo* manipulations, and at risk of accumulating pre-leukemic mutations, the effect of which can inadvertently be increased by gene correction.<sup>73,74</sup> On the other hand, the disease-specific challenges can also be viewed as opportunities. An example is lipoprotein lipase deficiency, in which enzyme replacement therapy is not effective, but intramuscular injection of the enzyme gene is, and the European Union's approval of alipogene tiparvovec for therapy of this disorder. This is correctly seen as encouraging news for all of gene therapy.<sup>75</sup> In this way, using these technologies in a rare and narrow disorder leads the way for other uses to follow more easily.

In all clinical applications of the gene corrective strategy, the cell type manipulated and delivery of gene-corrected cells has to be done in a disease-specific fashion. For example, in another clinically significant development, the immune system and its responses can be manipulated by transgenic means. In this book, three chapters deal with different aspects of the intersection of lymphohematopoiesis and gene therapy: Yang and Chan summarize efforts in gene therapy for diabetes; Gill and Kalos describe the great potential of T cell-based anticancer approaches; and Chen, Xie, Wu, and Chan discuss the immune response to pancreatic beta cells in type 1 diabetes and immunotherapies that can target this process. Related to this, Ning and Rabkin as well as Kratzke and Patel discuss the use of gene therapy with oncolytic viruses, induced suicide therapy, and immunotherapy for glioblastoma, one of the most lethal types of brain tumors.

## 1.6 ROADMAP TO THE FUTURE

These critical steps—the ability to modify the structure of DNA, the fact that all cells contain the blueprint for a complete organism, that cells can be regressed to a state of pluripotency, and that with appropriate immune manipulation cells from one individual can be transplanted into another—make it possible to transfer defined cells and designer genomes into whole organisms, thus completing the synthetic biology concept of stem cell gene therapy.

It should be noted that these discoveries were from areas of research almost entirely unconnected to each other: developmental biology, molecular crystallography, and tissue grafting. Individually, they answered important questions fundamental for their fields. Taken together, they complemented each other and represented a major turning point in biology and medicine. Furthermore, it was not obvious at that time how these milestones from three separate fields were related; as is often the case for any turning point in history, the consequences were recognizable only in retrospect. This may also be the case in the current development of gene therapy, in which critical discoveries are being made in different fields and by unconnected researchers.

Some areas of needed collaboration seem obvious. Improvements in vectorology, gene editing, and the delivery of the therapeutic genes into targeted cell populations need to connect with the development of other experimental therapies, such as the expansion of corrected cells with stem cell potential. These developments will likely result in patient-specific therapy, partial examples of which already exist. This indicates that an individualized medical approach is possible and that