REVIEW ARTICLE

GENOMIC MEDICINE

W. Gregory Feero, M.D., Ph.D., and Alan E. Guttmacher, M.D., *Editors*

New Therapeutic Approaches to Mendelian Disorders

Harry C. Dietz, M.D.

From the McKusick–Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore. Address reprint requests to Dr. Dietz at the McKusick–Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, 733 N. Broadway St., Baltimore, MD 21205, or at hdietz@jhmi.edu.

N Engl J Med 2010;363:852-63.

Progress in the treatment of genetic disorders has derived from insight into their causes and has focused on nutritional limitation of a substrate, the purging of a toxic metabolite, or compensatory expression of a protein whose deficiency causes disease, through gene delivery. In this review, I focus on therapeutic strategies that exploit a precise understanding of the pathogenesis of a mendelian disease, giving examples that illustrate the strengths and limitations of each approach, as well as the potential for broadening its application to the treatment of more common disorders.

Replacement of Deficient Gene Products

The introduction of genetic material (with the use of gene therapy) to compensate for a deficiency has both intuitive appeal and evident obstacles — including delivery, achievement of sustained expression, avoidance of a deleterious immunologic or tissue-based response, and potential for inducing secondary disease based on gene disruption at the DNA insertion site arising after vectors are integrated. These topics have been discussed extensively in the literature and will not be a focus of this review. Recent reports of diseases treated through the replacement of deficient gene products with overt success include severe combined immunodeficiency caused by a deficiency of adenosine deaminase[1-3] and Leber's congenital amaurosis caused by mutations in the gene encoding retinal pigment epithelium-specific protein of 65 kD (*RPE65*).[4]

The concept of systemic delivery of a deficient enzyme to rescue cellular function in patients with lysosomal storage diseases derives from early cell-culture experiments by Neufeld and her group.[5] These investigators found that pairwise coculture of fibroblasts from patients with different forms of mucopolysaccharidosis (i.e., forms caused by mutations affecting different lysosomal enzymes) resulted in the correction of both metabolic defects on a cellular level (an event called complementation). The secreted mediators of complementation were in fact the "deficient" enzymes, each taken up by the fibroblasts with the deficiency and transported to lysosomes.

Early attempts at enzyme-replacement therapy (ERT) for lysosomal storage disorders in vivo resulted in variable and largely disappointing results. Insights came from the confluence of basic biology and, in parallel, medical inquiry. While studying a newly described type of mucolipidosis — mucolipidosis type II (also known as inclusion-cell disease) — Hickman and Neufeld[6] tried to reconcile observations made on the basis of cultures of cells obtained from patients. Multiple lysosomal hydrolases accumulated in the culture medium and, despite their abundance, did not complement other disease states. The prevailing hypothesis was that the defect

was related to an enzyme that modifies multiple lysosomal hydrolases, allowing them to reenter cells. This hypothesis turned out to be true: *N*-acetylglucosamine-1-phosphotransferase modifies acid hydrolases through the addition of phosphomannosyl residues, which serve as "recognition" markers for both the uptake and transport of the hydrolases to lysosomes — events mediated by the mannose-6-phosphate receptor.[7] The predominant uptake pathway varies according to cell type. For example, macrophages (an important therapeutic target in Gaucher's disease) make substantial use of the mannose and asialoglycoprotein receptors.

Knowledge of the biochemistry and pathway of lysosomal enzymes resulted in clinical trials showing that ERT attenuates at least some presentations of lysosomal storage disorders, followed by approval by the Food and Drug Administration (FDA) of agents used in ERT for six lysosomal storage diseases (Table 1).

The biologic bases of variation in the safety and efficacy of ERT are both universal and specific to the individual patient. For example, treatment involving modifications of enzymes (e.g., through the addition of mannose-6-phosphate) to target certain tissues (e.g., the heart and kidney) is ill suited to targeting others (e.g., skeletal muscle) that have inherently poor uptake potential owing to low, or no, expression of the relevant receptor. Phenotype–genotype correlations also need to be considered. Disease alleles that cause a profound paucity of enzyme expression can promote an immunologic response against ERT that limits the efficacy of the treatment.[8] Mutations with severe effect can promote the involvement of the central nervous system (as in Gaucher's disease type 2 or 3) — a difficult scenario, given the inability of current targeting strategies used in ERT to bypass the blood–brain barrier. Emerging strategies to mitigate these limitations include the use of immune tolerance regimens,[8,9] modified targeting procedures,[10,11] or complementary therapeutic methods (e.g., those involving pharmacologic chaperones or substrate-reducing agents) (Fig. 1).

COMPENSATION FOR FUNCTIONAL DEFICITS THROUGH NEW APPLICATIONS OF FDA-APPROVED DRUGS

The Hutchinson–Gilford progeria syndrome has an onset in childhood and is characterized by rapid progression of clinical features resembling aging, including alopecia, loss of subcutaneous fat, aggressive atherosclerosis, and degeneration of the joints, bone, and skin.[12] Patients die from vascular disease, generally before 20 years of age. The disease is caused by mutations of the *LMNA* gene, encoding lamin A (a structural protein of the nuclear membrane).[13] A common de novo mutation (see Glossary) results in progerin, a mutant form of pre–lamin A that harbors a deletion of 50 amino acids. Progerin is targeted to the nuclear membrane by means of a farnesyl group that is bound to its C-terminal. Subsequent cleavage of progerin by the zinc metalloproteinase ZMPSTE24 releases mature lamin A, which goes on to participate in a variety of nuclear functions. Progerin, in contrast, lacks the cleavage site and remains tethered to the nuclear membrane (Fig. 2). On the basis of this information, attention turned to a class of drugs called farnesyl transferase inhibitors, initially developed for cancer treatment. The hope was that inhibiting the farnesylation of progerin would prevent the transport of progerin and hence its irreversible sequestration at the nuclear membrane (Fig. 2).

Nuclear blebbing (characterized by small protuberances of the nuclear membrane) is observed in cultured cells from patients with the Hutchinson–Gilford progeria syndrome. Young and his group[14] showed that, after treatment with farnesyltransferase inhibitors, cells from unaffected persons and cells from patients with the Hutchinson–Gilford progeria syndrome mislocalized pre–lamin A and progerin, respectively, away from the nuclear membrane, and the cells from the patients showed reduced nuclear blebbing. Treatment with farnesyltransferase inhibitors also improved growth, fat mass, and bone mineralization in mice carrying a mutation that causes the Hutchinson–Gilford progeria syndrome.[15] In this mouse model of the syndrome, vascular disease does not develop. Affected mice die prematurely, and the influence of farnesyltransferase inhibitors on survival was not assessed. Collins and his group[16] tested the farnesyltransferase inhibitors tipifarnib (Zarnestra) in a transgenic mouse model of the Hutchinson–Gilford progeria syndrome that has vascular disease of the aortic media; the use of tipifarnib resulted in a protective effect on disease onset and progression proportionate to the degree of inhibition of farnesylation. Taken together, these data suggest that farnesyltransferase inhibi-

Table 1. Established and Investigational Therapies for Lysosomal Storage Diseases.*

Disease	Enzyme Replaced or Targeted	Therapeutic Agent†	Manufacturer	Indication	Status of Agent
Commercially available therapies					
Gaucher's disease type 1	Glucocerebrosidase	Imiglucerase (Cerezyme)	Genzyme	ERT	FDA approved
Gaucher's disease type 1	Glucocerebrosidase	Miglustat (Zavesca)	Actelion	SRT	FDA approved
Fabry's disease	α-Galactosidase A	Agalsidase beta (Fabrazyme)	Genzyme	ERT	FDA approved
Pompe's disease	α-Glucosidase	Alglucosidase alfa (Myozyme)	Genzyme	ERT	FDA approved
MPS II (Hunter's syndrome)	Iduronate-2-sulfatase	Idursulfase (Elaprase)	Shire	ERT	FDA approved
MPS VI (Maroteaux–Lamy syndrome)	Arylsulfatase B	Galsulfase (Naglazyme)	BioMarin	ERT	FDA approved
MPS I (Hurler's syndrome or the Hurler–Scheie syndrome)	α-L-iduronidase	Laronidase (Aldurazyme)	BioMarin–Genzyme	ERT	FDA approved
Gaucher's disease type 1	Glucocerebrosidase	Velaglucerase alfa	Shire	ERT	FDA approved
Investigational therapies					
Gaucher's disease type 1	Glucocerebrosidase	Taliglucerase alfa (Uplyso)	Protalix	ERT	In phase 3 study
Gaucher's disease type 1	Glucocerebrosidase	Isofagomine tartrate (Plicera)	Amicus	Pharmacologic chaperone	In phase 2 study
Fabry's disease	α-Galactosidase A	Migalastat hydrochloride (Amigal)	Amicus	Pharmacologic chaperone	In phase 2 study
Fabry's disease	α-Galactosidase A	Agalsidase alfa (Replagal)	Shire	ERT	In phase 3 study (approved in EU)
Pompe's disease	α-Glucosidase	AT2220	Amicus	Pharmacologic chaperone	In phase 2 study
Niemann–Pick disease type C	Sphingomyelinase	Miglustat (Zavesca)	Actelion	SRT	In phase 2 study (approved in EU)
Tay–Sachs disease	Hexosaminidase A	Miglustat (Zavesca)	Actelion	SRT	In phase 2 study

* ERT denotes enzyme-replacement therapy, EU European Union, FDA Food and Drug Administration, MPS mucopolysaccharidosis, and SRT substrate-reduction therapy.
† Therapeutic agents are listed by their U.S. adopted name followed by the trade name (if any) in parentheses.

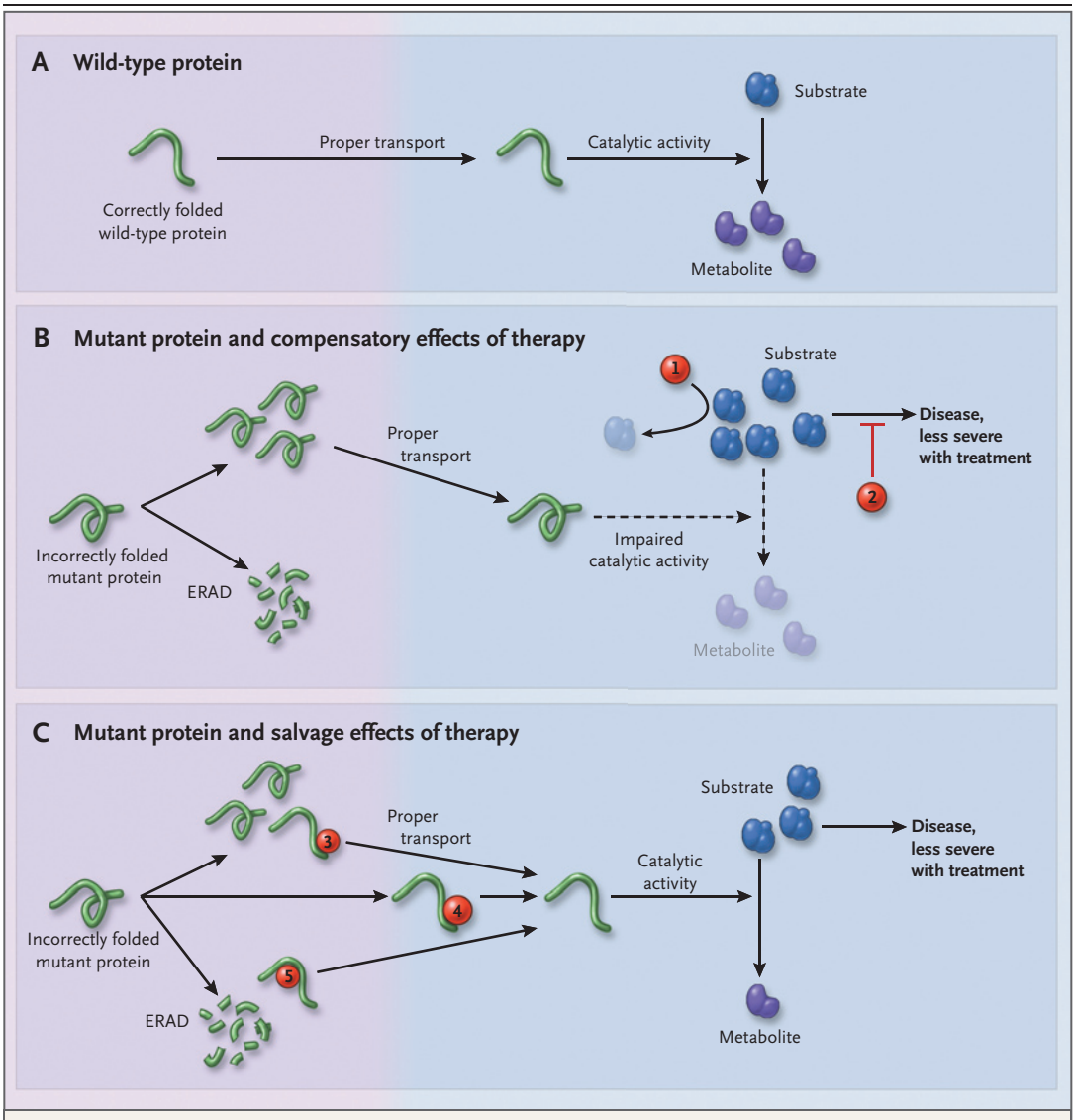

Figure 1. Compensatory and Salvage Mechanisms of Action of Therapeutic Agents.

Wild-type protein (Panel A) folds correctly and is properly transported from the site of synthesis (e.g., the endoplasmic reticulum [ER]) to the site of intended function (e.g., the lysosome) and performs its intended function (e.g., the catalysis of hypothetical substrate A to hypothetical metabolite B). Mutant forms of protein (Panels B and C) can fold improperly, leading to failed transport from the site of synthesis, proper transport but impaired catalytic activity, or ER-associated degradation (ERAD). All these events will result in accumulation of substrate, which could initiate events that culminate in disease. Therapeutic gain could result from the use of various types of drugs (red circles). Compensatory effects of therapy (Panel B) include the possibility that drugs (represented by drug 1) can achieve clearance of the substrate by means of alternative mechanisms (substrate-reduction therapy) and other drugs (represented by drug 2) can antagonize pathogenetic events downstream. Salvage effects of therapy (Panel C) include the possibility that any of the three types of drugs can act as pharmacologic chaperones to mediate improvement in the folding of mutant proteins, which could mitigate the phenotypic severity of disease by means of the rescue of transport (by corrector drugs [3]), intrinsic catalytic activity (by potentiator drugs [4]), or stability (by stabilizer drugs [5]).

tors can attenuate progression of the phenotypes of the Hutchinson–Gilford progeria syndrome, justifying the launch of a clinical trial.

Questions remain. Because alterations in nuclear morphologic characteristics reminiscent of those in patients with the Hutchinson–Gilford progeria syndrome are also seen in patients with other conditions caused by mutations in *LMNA* that

Glossary

Antisense oligonucleotides: Short, synthetic single-stranded RNA or DNA molecules that bind to target RNA and induce their degradation or inhibit their processing or translation.

De novo mutation: Any DNA sequence change that occurs during replication, such as (in the context of this article) a heritable gene alteration occurring in a family for the first time as a result of a DNA sequence change in a germ cell or fertilized egg.

Exon–intron junction: A junction between a block of coding sequence (exon) and an adjacent block of noncoding sequence (intron) present in DNA and in precursor messenger RNA (pre-mRNA).

In-frame exon skipping: The skipping of an exon that contains a multiple of three nucleotides during splicing of pre-mRNA, resulting in the preservation of the reading frame for translation.

Kinase: An enzyme that transfers a phosphate group to a substrate.

MicroRNA: A short regulatory form of RNA that binds to a target RNA molecule and generally suppresses its translation by ribosomes.

Nonsense-mediated mRNA decay: The process by which cells recognize and decay mRNA that encodes a premature signal for the termination of translation.

Small (or short) interfering RNA: A short, single-stranded regulatory RNA molecule that binds to and induces the degradation of target RNA molecules.

Translational readthrough: Bypass of a termination codon by the ribosome after incorporation of a random amino acid into the elongating peptide.

neither alter processing of pre–lamin A nor are associated with premature features of aging, it remains unclear whether nuclear blebbing is an informative surrogate for critical phenotypes of the Hutchinson–Gilford progeria syndrome. Moreover, it is possible that more relevant pathogenic mechanisms depend on an inability of progerin to support the essential nuclear functions carried out by mature (processed) lamin A.

Marfan's syndrome is a connective-tissue disorder caused by mutations in the *FBN1* gene encoding the extracellular matrix protein fibrillin 1. Patients show a severe deficiency of fibrillin 1 aggregates (microfibrils) that would otherwise instruct the formation and homeostasis of elastic fibers. It was therefore hypothesized that disease manifestations (e.g., aortic aneurysm, pulmonary emphysema, eye-lens dislocation, and stretch marks on the skin) reflect a simple structural weakness of the tissues.

However, other manifestations of Marfan's syndrome — such as bone overgrowth and muscle hypoplasia — are not so easily explained. An analysis of mouse models of Marfan's syndrome has shown that microfibrils normally bind the large latent complex of the cytokine transforming growth factor β (TGF-β) and that failure of this event to occur results in increased TGF-β activation and signaling.[17] Moreover, antagonism of TGF-β signaling attenuates or prevents many disease manifestations — including aneurysm, emphysema, myxomatous degeneration of heart valves, and skeletal-muscle myopathy — in mouse models of Marfan's syndrome.[18]

Previous work had shown that angiotensin II signaling increases TGF-β activity in mouse models of chronic kidney disease and that this effect can be blocked with the use of losartan, an FDA-approved antagonist of the angiotensin II type 1 receptor that is widely used to treat hypertension. Oral administration of losartan in a mouse model of Marfan's syndrome prevented aortic-root enlargement and improved aortic-wall architecture through productive remodeling.[19] Losartan also addressed manifestations outside the vascular system, including emphysema and myopathy.[18] On this basis, the Pediatric Heart Network launched a clinical trial of losartan (ClinicalTrials.gov number, NCT00429364) in patients with Marfan's syndrome.[20] Although this trial has yet to be concluded, my colleagues and I[21] treated a subgroup of children with severe and rapidly progressive Marfan's syndrome, typically leading to aortic surgery or death in early childhood, and found a reduction in the rate of aortic-root growth after the initiation of losartan therapy to approximately 10% of the rate seen in the same cohort previously (while receiving a different type of medical therapy). Both people and mice with Marfan's syndrome have elevated plasma levels of TGF-β that decrease with the use of losartan, and the level correlates with aortic size in mice.[22] These data suggest that circulating TGF-β may serve as a prognostic and therapeutic marker in patients

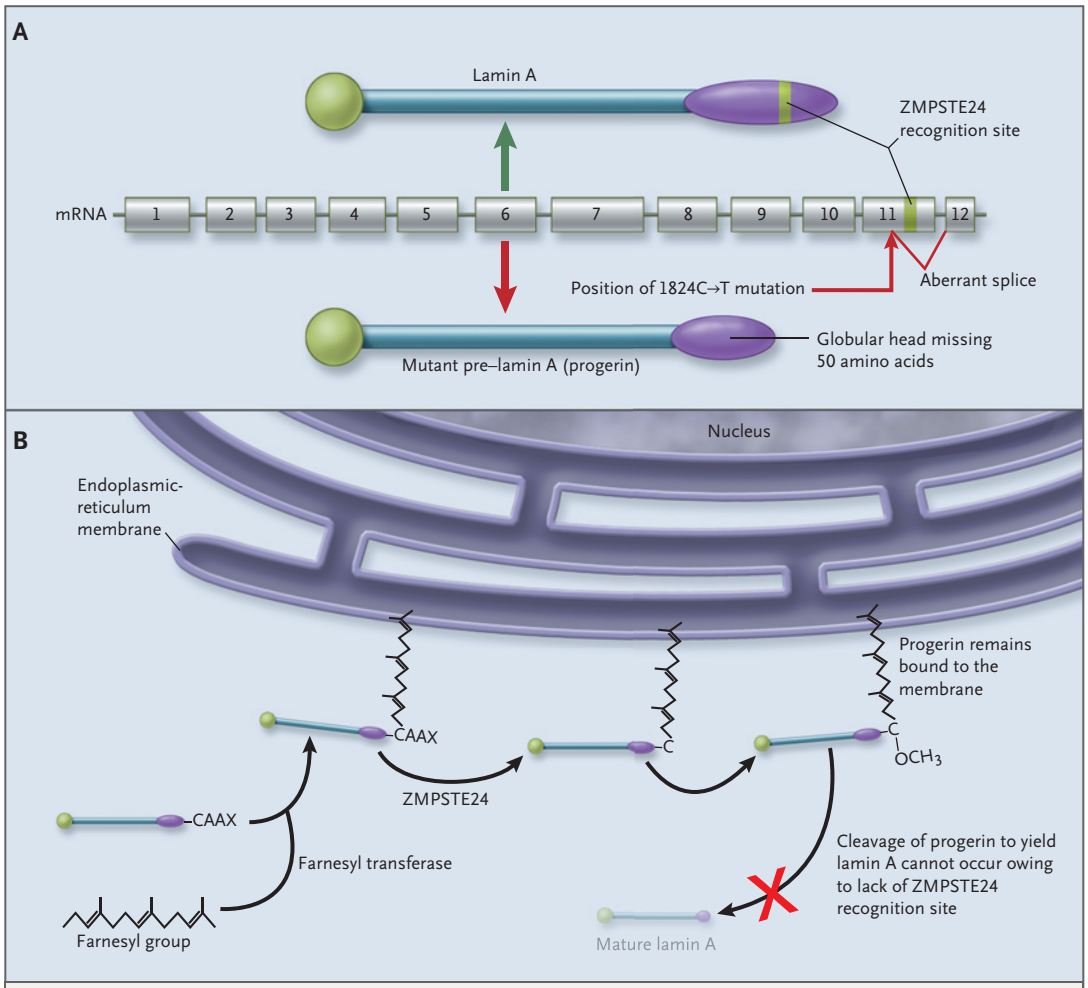

Figure 2. Proposed Pathogenesis of the Hutchinson–Gilford Progeria Syndrome.

The *LMNA* gene, encoding lamin A (a structural protein of the nuclear membrane), contains 12 exons (Panel A). Normally, spliced pre–messenger RNA (mRNA) results in lamin A that encodes a recognition and cleavage site for the enzyme ZMPSTE24 (red bar) within the C-terminal globular domain (blue oval). The 1824C→T mutation in exon 11 is found in most people with Hutchinson–Gilford progeria syndrome. The mutation activates a cryptic splice donor, causing an abnormal splicing event that removes 150 nucleotides from the mRNA and hence 50 amino acids that span the ZMPSTE24 cleavage site in the C-terminal globular domain. This results in a mutant form of pre–lamin A (progerin) that cannot undergo C-terminal processing. As it does to lamin A, farnesyl transferase (Panel B) adds a farnesyl group to the extreme C-terminal of progerin, at a cysteine residue (indicated with the letter C) within the so-called terminal CAAX motif (with the letter A indicating an aliphatic amino acid; and X, any amino acid). The farnesyl group binds the lamin A or progerin to the membrane of the endoplasmic reticulum at the periphery of the nucleus, where ZMPSTE24 cleaves the three terminal amino acids (AAX) of both proteins; the farnesylated cysteine is then carboxymethylated (i.e., an OCH_3 group is added). Lamin A undergoes a second cleavage event by ZMPSTE24, within the C-terminal globular domain, releasing mature lamin A. Progerin remains farnesylated and tethered to the membrane, because the abnormal splicing event has deleted this cleavage site.

with Marfan's syndrome, allowing for tailoring of therapeutic regimens to the individual patient.

Ongoing efforts are aimed at identifying events parallel to or downstream of TGF-β signaling that could serve as therapeutic targets. For example, TGF-β can induce the activity of matrix-degrading enzymes such as matrix metalloproteinases 2 and 9. Preliminary evidence suggests that the matrix-metalloproteinase antagonist doxycycline can attenuate aortic-root growth in mouse models of Marfan's syndrome.[23,24]

TGF-β antagonism shows potential as a therapy for other genetically induced forms of aortic aneurysm. Indeed, increased aortic-wall TGF-β

signaling has been observed in patients with various conditions, including the Loeys–Dietz syndrome (an aggressive vasculopathy caused by mutations in *TGFBR1* or *TGFBR2*, genes encoding the TGF-β receptor),[25,26] cutis laxa with aneurysm,[27] the arterial tortuosity syndrome,[28] and bicuspid aortic valve with aneurysm.[29]

SMALL MOLECULES AS THERAPEUTIC AGENTS

A path to effective drug therapy for a genetic disorder that highlights both the requirements and potential of using small molecules as therapeutic agents is exemplified by the development of imatinib (Gleevec) for the treatment of chronic myelogenous leukemia.[30] Three milestones in particular marked the road to its approval by the FDA in 2001. First, an abnormally short chromosome (the so-called Philadelphia chromosome), later shown to result from a translocation between chromosomes 22 and 9, was found to be associated with chronic myelogenous leukemia. Second, the translocation was shown to result in a fusion transcript derived from the breakpoint cluster region gene (*BCR*) and the gene encoding the nonreceptor tyrosine kinase c-ABL (known to be homologous to the viral oncogene v-abl) and that the resultant fusion protein (BCR-ABL) had elevated and constitutive kinase activity sufficient to transform cells into leukemia. The third milestone was the implementation of small-molecule screening assays to identify compounds that specifically inhibit the activity of tyrosine kinases. These studies culminated in successful clinical trials of imatinib use in patients. This research was performed in an incremental and deliberate manner, and it resulted in a drug screening assay honed to address the central pathogenetic event of the disease (i.e., its Achilles' heel).[31]

The desirable functions of small-molecule therapeutic compounds vary according to circumstances and can be subdivided into broad classes (Fig. 1). Pharmacologic chaperones generally bind directly to mutant proteins and can counteract aspects of cellular "quality-control" systems that serve to maintain the structural precision of proteins but may be nonproductive in the context of disease.[32] Pharmacologic "correctors" and "stabilizers" can result in restored transportability and stability, respectively, and hence elevated levels of mutant proteins that retain residual function. "Potentiators" improve the inherent functional capacity of mutant proteins. The rescue of cellular or tissue function by a pharmacologic compound can also relate to direct inhibition of a toxic function (e.g., imatinib use in patients with chronic myelogenous leukemia to inhibit dysregulated kinase activity) or indirect compensation for perturbation of a critical function (e.g., clearance of toxic metabolites).

Some relevant principles of substrate reduction are illustrated through consideration of Gaucher's disease, which is caused by the tissue accumulation of glycosylceramide due to a deficiency of glucocerebrosidase activity. Limitations of ERT (particularly in treating the neuronopathic type 2 or 3 form of Gaucher's disease) have provided incentives for complementary and alternative therapies. First conceived as a means to reduce the substrate for glucocerebrosidase,[33] attention turned to the *N*-alkylated iminosugar *N*-(*n*-butyl)deoxynojirimycin (miglustat; previously known as NB-DNJ), an inhibitor of glycosylceramide synthase that crosses the blood–brain barrier. In clinical use, miglustat has proved effective in the management of the hematologic and bone manifestations and organomegaly of Gaucher's disease type 1 in patients in whom ERT is unsuitable.[34-37] Early results regarding miglustat therapy for neuronopathic manifestations of Gaucher's disease have been less encouraging.[38] Given that glycosylceramide is also the precursor for several glycosphingolipids, miglustat is also being explored as a treatment for other diseases, including GM1 and GM2 gangliosidosis (e.g., Niemann–Pick disease and Tay–Sachs disease).

Miglustat and other iminosugar derivatives such as *N*-(*n*-nonyl)deoxynojirimycin (or NN-DNJ) and isofagomine are also pharmacologic chaperones for certain mutant forms of glucocerebrosidase. The binding of miglustat to the active site of glucocerebrosidase inhibits the activity of the enzyme at cellular pH but stabilizes the mutant protein through conformational change and, hence, avoidance of degradation pathways. Enhanced enzymatic activity can be seen at subinhibitory concentrations of miglustat or on dissociation after transport to the more acidic environment of the lysosome. This counterintuitive approach to therapy, involving the use of an active-site inhibitor to restore activity, has been applied in other contexts.[39] Other experimental strategies to treat Gaucher's disease include the use of calcium-channel

blockers as molecular chaperones or proteostasis regulators to increase the concentration of the mutant enzyme that has the ability to be transported.[40-42]

MANIPULATION OF GENE EXPRESSION — NONSENSE SUPPRESSION

A premature signal for the termination of translation can be caused by a single nucleotide substitution that creates a premature-termination codon or the deletion or insertion of nucleotides that shifts the messenger RNA (mRNA) reading frame (i.e., a frameshift mutation) (Fig. 3). Premature-termination codons that occur more than 50 nucleotides upstream of the final exon–intron junction generally induce transcript degradation through the nonsense-mediated mRNA decay surveillance pathway (Fig. 3).[43] Truncated proteins that derive from any residual nonsense transcripts typically lack their intended function. In 1985, Burke and Mogg[44] showed that aminoglycoside antibiotics can suppress the effect of a nonsense mutation (an action termed "nonsense suppression") by inducing the incorporation of a random amino acid at a premature-termination codon in mammalian cells (Fig. 3). The "stop" mutation is thus effectively ignored by the translational apparatus. Bedwell and colleagues[45,46] later showed that aminoglycosides can increase the expression and function of the full-length cystic fibrosis transmembrane conductance regulator protein (CFTR) in cultured respiratory epithelial cells, derived from patients with cystic fibrosis, that have nonsense alleles. This phenomenon was replicated in cell lines obtained from patients with other diseases[47,48]; more variable results were seen in mouse models of Duchenne's muscular dystrophy[49,50] and in patients with either Duchenne's muscular dystrophy[51,52] or cystic fibrosis.[53,54]

A screening assay to identify new agents with potent nonsense-suppression activity resulted in the identification of PTC124 as a safe, nonaminoglycoside suppressor with oral bioavailability.[55] PTC124 was shown to increase the expression of full-length protein in mouse models of Duchenne's muscular dystrophy[56] and cystic fibrosis[57] and to improve CFTR expression and function in nasal-epithelium specimens obtained from a subgroup of patients with cystic fibrosis.[58]

Despite the justified excitement about a potential therapy pertinent to many genetic diseases, potential limitations also need to be considered. First, nonsense suppressors do not appear to counteract nonsense-mediated mRNA decay, greatly limiting the number of nonsense transcripts available for translation. Some studies showing efficacy in animal models have used complementary DNA–based minigenes that lack introns and thus do not properly model the molecular pathogenic event, since pre-mRNA splicing is a requirement for mammalian nonsense-mediated mRNA decay. Studies of yeast have suggested that both the inhibition of nonsense-mediated mRNA decay and nonsense suppression ("translational readthrough," or bypass of a termination codon by the ribosome after incorporation of a random amino acid) are required for the rescue of cellular function.[59] Moreover, studies of yeast suggest that transcripts that have undergone nonsense surveillance but escape degradation are not efficiently translated.[60] Second, the incorporation of any random amino acid at a premature-termination codon may not be sufficient to restore protein function, even if a full-length protein is generated, if the intended amino acid at this position (as specified by the wild-type sequence) is critical. Third, the efficiency of readthrough for all nonsense suppressors (including PTC124) is highly dependent on the nature of the "stop" codon and its broader sequence context, greatly limiting the number of nonsense alleles that can be targeted by this therapy. Fourth, readthrough of physiologic termination codons could be detrimental if it leads to the use of an alternative mechanism of transcript clearance (nonstop decay)[61] or the creation of proteins with an extended C-terminal. Finally, the apparent "readthrough" properties of PTC124 may relate, at least in part, to an artifact in the screening assay used to identify readthrough.[62]

MANIPULATION OF PRE-MRNA SPLICING

Short oligonucleotides show high potential for therapeutic applications. These oligonucleotides include small interfering RNA (siRNA) and microRNA used to degrade mRNA transcripts and suppress protein translation, respectively, and antisense oligonucleotides used to manipulate splicing.

Many mutant dystrophin alleles causing Duchenne's muscular dystrophy harbor nonsense or frameshift mutations that preclude protein expres-

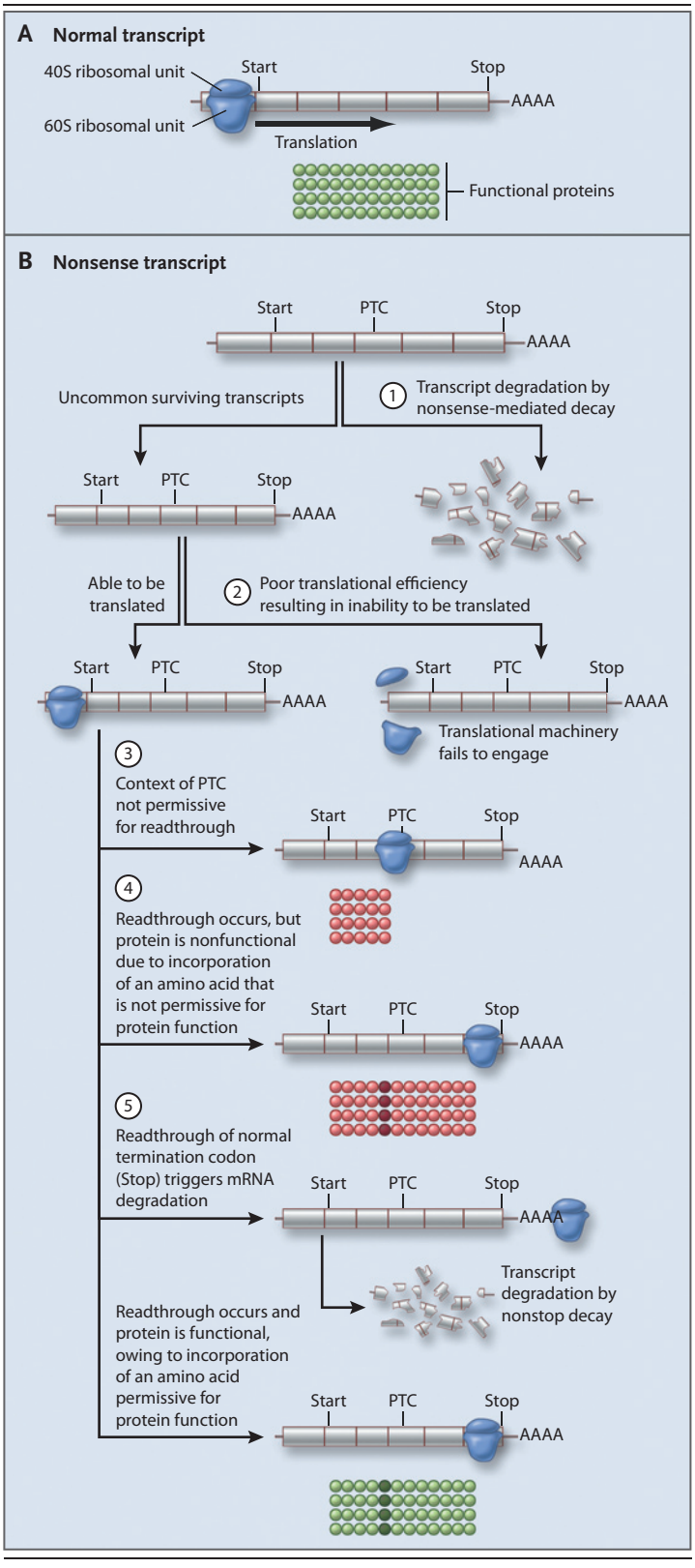

Figure 3. Potential and Pitfalls of Therapeutic Strategies Aimed at Promoting Readthrough of Nonsense Transcripts Containing a Premature-Termination Codon (PTC).

A hypothetical normal messenger RNA (mRNA) molecule containing six exons is used to show the potentials and pitfalls of therapeutic strategies involving nonsense suppression. Panel A shows that the start site for translation, in exon 2, engages the 40S and 60S ribosomal subunits (blue ovals) to initiate translation. The mature mRNA has undergone terminal processing with the addition of a string of adenosines (indicated with the letter A) (the poly-A tail). Translation from the start codon to the normal stop codon at the end of exon 6 results in the formation of functional protein (designated by strings of amino acids [green circles]). Panel B shows the potential fate of a nonsense transcript harboring a PTC in exon 4 (step 1). Most PTCs upstream of the distal portion of the penultimate exon will initiate transcript degradation by means of nonsense-mediated mRNA decay. The few transcripts that escape nonsense-mediated mRNA decay may be incompetent to initiate translation (step 2). Only the few remaining transcripts that are competent for translational initiation can be acted on by a readthrough-promoting agent. However, many PTCs occur within a context that is not permissive for readthrough, despite the use of a drug, resulting in truncated and nonfunctional protein (designated by short strings of amino acids [red circles]) (step 3). If readthrough occurs, the random incorporation of any amino acid (colored dark red) at a site where the intended amino acid has critical function would also result in nonfunctional protein (step 4). Robust readthrough could allow the ribosome to bypass both the PTC and normal stop codons, triggering other mechanisms for transcript degradation, such as nonstop decay (step 5). If all goes well, an amino acid permissive for protein function (colored dark green) will be incorporated at the PTC, and translational termination will occur at the appropriate site, resulting in the production of full-length, functional protein at levels sufficient to mitigate or prevent disease.

sion due to nonsense-mediated mRNA decay. In contrast, many dystrophin alleles causing the more mild Becker's muscular dystrophy involve the in-frame skipping of central exons, allowing for the production of dystrophin molecules that retain some residual function even though they lack a central segment. This finding suggests that induced skipping of an exon that either harbors a mutation or compensates for a frame shift should attenuate phenotypic severity. (That is, enforced skipping of such an exon might ameliorate Duchenne's muscular dystrophy so that it more closely resembles Becker's muscular dystrophy.)

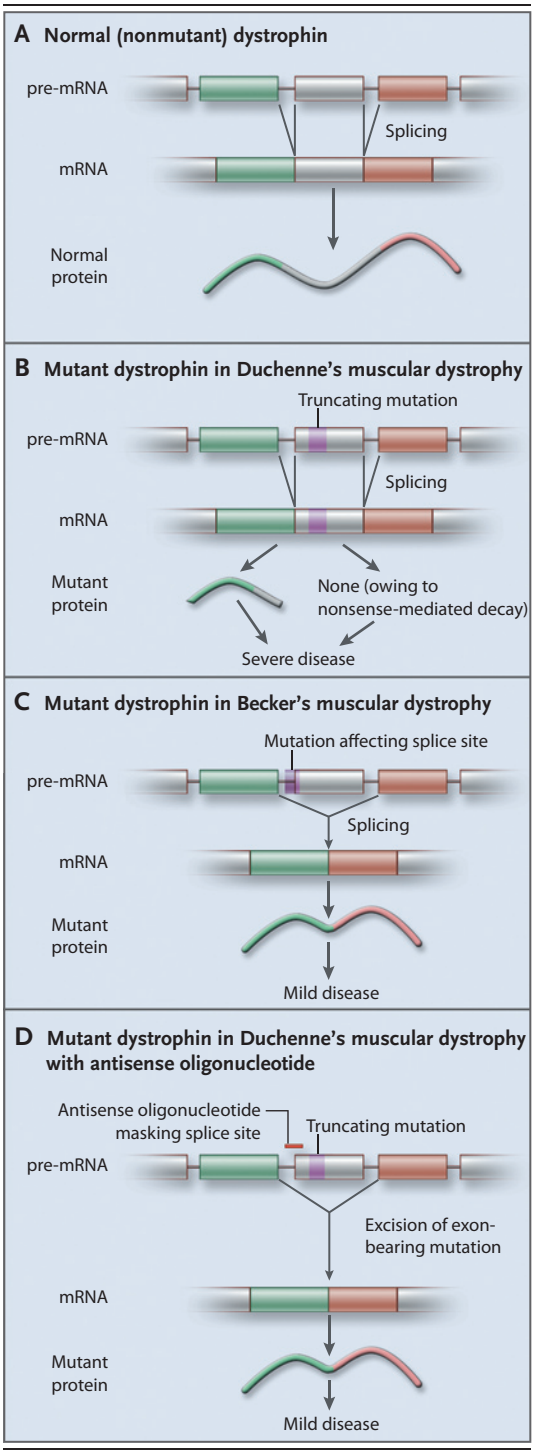

Figure 4. Induced Exon Skipping in the Treatment of Duchenne's Muscular Dystrophy.

Normal dystrophin pre–messenger RNA (mRNA) (only an internal segment is shown) undergoes normal splicing and results in normal dystrophin mRNA and protein (Panel A). Duchenne's muscular dystrophy (Panel B) is usually caused by premature-termination-codon mutations that either induce transcript clearance by means of nonsense-mediated mRNA decay or lead to the formation of a truncated, nonfunctional protein. A milder form of disease, called Becker's muscular dystrophy (Panel C), is often caused by splice-site mutations that induce the skipping of an internal exon that is a multiple of three nucleotides in length during dystrophin pre-mRNA splicing. The resulting mRNA is lacking its central segment but retains an open reading frame for translation. This maintains transcript stability and allows for the production of a form of dystrophin protein that retains some residual function. The introduction of a synthetic antisense oligonucleotide (Panel D, purple bar) that binds to a splice junction of an exon containing a premature-termination codon in a patient with a typical mutation associated with Duchenne's muscular dystrophy can prevent recognition of that exon by the splicing machinery, resulting in exon skipping. By removing the premature-termination codon from the mRNA, this strategy has the proven potential to maintain stability of the mutant transcript and to allow for the production of a dystrophin protein retaining some residual function. In theory, this would result in the milder phenotype of Becker's muscular dystrophy in a patient otherwise destined to have Duchenne's muscular dystrophy.

The strategy of enforced skipping involves the uptake of antisense oligonucleotides that target splice junctions, precluding their recognition by the splicing machinery (Fig. 4). Various modifications of antisense oligonucleotides to promote their stability, uptake, and extent to which they promote exon skipping have proved to result in the successful expression of productive proteins, both in cell culture and after local delivery to animal models[63-65] and people with Duchenne's muscular dystrophy.[66] Intravascular injection of antisense oligonucleotides has improved dystrophin expression in skeletal muscle and muscle function in mice and dogs with muscular dystrophy; the efficacy with which antisense oligonucleotides can be targeted to cardiac muscle is variable, with increased efficacy seen in association with more recent modifications to antisense oligonucleotides.[67]

There are many potential limitations of this approach, including the difficulty of delivering antisense oligonucleotides, the short duration of their effect, and the diversity of mutations in the large dystrophin gene associated with Duchenne's muscular dystrophy, which may necessitate extensive optimization of the therapy for each patient. Fortunately, it seems that a limited repertoire of antisense oligonucleotides or cocktails thereof can induce compensation by means of single or multiple exon-skipping events that address the bulk

of the mutations underlying Duchenne's muscular dystrophy, according to an international database.[68,69]

CONCLUSIONS

Continued efforts to develop therapies for mendelian disorders represent both an obligation and an opportunity. The obligation relates to the fact that although mendelian diseases are individually rare, they are quite common when viewed as a group, and their burden on patients is great. In addition, to a large extent, patients with rare genetic disorders have fueled progress in the fields of human genetics and molecular therapeutics through their enthusiastic participation in research, often based on a remote promise of personal gain and at a very real personal expense. The opportunity relates to the very nature of mendelian diseases; the single-gene basis of the defect underlying each mendelian disease implies genes and pathways that are sufficient to cause phenotypes of interest and are therefore inherently able to be targeted for purposes of therapeutic gain, even when the phenotype occurs in patients with a more common presentation and a more complex cause.

The primary identification of genetic susceptibility loci, by means of genomewide association studies for complex traits, has provided clues to the pathogenesis of these diseases and therapies for them[70-72] and will undoubtedly continue to do so. The realization of such therapies will be facilitated, in part, by using knowledge gained from research into mendelian disorders to sort and prioritize the leads emerging from genomewide association studies.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

REFERENCES

1. Aiuti A, Brigida I, Ferrua F, et al. Hematopoietic stem cell gene therapy for adenosine deaminase deficient-SCID. Immunol Res 2009;44:150-9.
2. Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009;360:447-58.
3. Booth C, Hershfield M, Notarangelo L, et al. Management options for adenosine deaminase deficiency: proceedings of the EBMT satellite workshop (Hamburg, March 2006). Clin Immunol 2007;123:139-47.
4. Cideciyan AV, Hauswirth WW, Aleman TS, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. Hum Gene Ther 2009;20:999-1004.
5. Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. Science 1968;162:570-2.
6. Hickman S, Neufeld EF. A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. Biochem Biophys Res Commun 1972;49:992-9.
7. Kornfeld S. Trafficking of lysosomal enzymes in normal and disease states. J Clin Invest 1986;77:1-6.
8. Ponder KP. Immune response hinders therapy for lysosomal storage diseases. J Clin Invest 2008;118:2686-9.
9. Kakkis E, Lester T, Yang R, et al. Successful induction of immune tolerance to enzyme replacement therapy in canine mucopolysaccharidosis I. Proc Natl Acad Sci U S A 2004;101:829-34.
10. Rapoport M, Lorberboum-Galski H. TAT-based drug delivery system — new directions in protein delivery for new hopes? Expert Opin Drug Deliv 2009;6:453-63.
11. Begley DJ, Pontikis CC, Scarpa M. Lysosomal storage diseases and the blood-brain barrier. Curr Pharm Des 2008;14:1566-80.
12. Merideth MA, Gordon LB, Clauss S, et al. Phenotype and course of Hutchinson–Gilford progeria syndrome. N Engl J Med 2008;358:592-604.
13. Eriksson M, Brown WT, Gordon LB, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 2003;423:293-8.
14. Toth JI, Yang SH, Qiao X, et al. Blocking protein farnesyltransferase improves nuclear shape in fibroblasts from humans with progeroid syndromes. Proc Natl Acad Sci U S A 2005;102:12873-8.
15. Yang SH, Qiao X, Fong LG, Young SG. Treatment with a farnesyltransferase inhibitor improves survival in mice with a Hutchinson-Gilford progeria syndrome mutation. Biochim Biophys Acta 2008;1781:36-9.
16. Capell BC, Olive M, Erdos MR, et al. A farnesyltransferase inhibitor prevents both the onset and late progression of cardiovascular disease in a progeria mouse model. Proc Natl Acad Sci U S A 2008;105:15902-7.
17. Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 2003;33:407-11.
18. Judge DP, Dietz HC. Therapy of Marfan syndrome. Annu Rev Med 2008;59:43-59.
19. Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006;312:117-21.
20. Lacro RV, Dietz HC, Wruck LM, et al. Rationale and design of a randomized clinical trial of beta-blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. Am Heart J 2007;154:624-31.
21. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC III. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med 2008;358:2787-95.
22. Matt P, Schoenhoff F, Habashi J, et al. Circulating transforming growth factor-beta in Marfan syndrome. Circulation 2009;120:526-32.
23. Chung AW, Yang HH, Radomski MW, van Breemen C. Long-term doxycycline is more effective than atenolol to prevent thoracic aortic aneurysm in Marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9. Circ Res 2008;102(8):e73-e85.
24. Xiong W, Knispel RA, Dietz HC, Ramirez F, Baxter BT. Doxycycline delays aneurysm rupture in a mouse model of Marfan syndrome. J Vasc Surg 2008;47:166-72.
25. Loeys BL, Schwarze U, Holm T, et al. Aneurysm syndromes caused by mutations in the TGF-β receptor. N Engl J Med 2006;355:788-98.
26. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet 2005;37:275-81.
27. Hanada K, Vermeij M, Garinis GA, et al. Perturbations of vascular homeostasis and aortic valve abnormalities in fibulin-4 deficient mice. Circ Res 2007;100:738-46.
28. Coucke PJ, Willaert A, Wessels MW, et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis

and cause arterial tortuosity syndrome. Nat Genet 2006;38:452-7.
29. Gomez D, Al Haj Zen A, Borges LF, et al. Syndromic and non-syndromic aneurysms of the human ascending aorta share activation of the Smad2 pathway. J Pathol 2009;218:131-42.
30. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood 2008;112:4808-17.
31. Druker BJ, Lydon NB. Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 2000;105:3-7.
32. Conn PM, Janovick JA. Drug development and the cellular quality control system. Trends Pharmacol Sci 2009;30:228-33.
33. Radin NS. Treatment of Gaucher disease with an enzyme inhibitor. Glycoconj J 1996;13:153-7.
34. Pastores GM, Giraldo P, Chérin P, Mehta A. Goal-oriented therapy with miglustat in Gaucher disease. Curr Med Res Opin 2009;25:23-37.
35. Hollak CE, Hughes D, van Schaik IN, Schwierin B, Bembi B. Miglustat (Zavesca) in type 1 Gaucher disease: 5-year results of a post-authorisation safety surveillance programme. Pharmacoepidemiol Drug Saf 2009;18:770-7.
36. Ficicioglu C. Review of miglustat for clinical management in Gaucher disease type 1. Ther Clin Risk Manag 2008;4:425-31.
37. Giraldo P, Alfonso P, Atutxa K, et al. Real-world clinical experience with long-term miglustat maintenance therapy in type 1 Gaucher disease: the ZAGAL project. Haematologica 2009;94:1771-5.
38. Schiffmann R, Fitzgibbon EJ, Harris C, et al. Randomized, controlled trial of miglustat in Gaucher's disease type 3. Ann Neurol 2008;64:514-22.
39. Fan JQ. A counterintuitive approach to treat enzyme deficiencies: use of enzyme inhibitors for restoring mutant enzyme activity. Biol Chem 2008;389:1-11.
40. Rigat B, Mahuran D. Diltiazem, a L-type Ca(2+) channel blocker, also acts as a pharmacological chaperone in Gaucher patient cells. Mol Genet Metab 2009; 96:225-32.
41. Mu TW, Fowler DM, Kelly JW. Partial restoration of mutant enzyme homeostasis in three distinct lysosomal storage disease cell lines by altering calcium homeostasis. PLoS Biol 2008;6(2):e26.
42. Mu TW, Ong DS, Wang YJ, et al. Chemical and biological approaches synergize to ameliorate protein-folding diseases. Cell 2008;134:769-81.
43. Frischmeyer PA, Dietz HC. Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet 1999;8:1893-900.
44. Burke JK, Mogg AE. Suppression of a nonsense mutation in mammalian cells in vivo by the aminoglycoside antibiotics G-418 and paromomycin. Nucleic Acids Res 1985;13:6265-72.
45. Howard M, Frizzell RA, Bedwell DM. Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. Nat Med 1996;2:467-9.
46. Bedwell DM, Kaenjak A, Benos DJ, et al. Suppression of a CFTR premature stop mutation in a bronchial epithelial cell line. Nat Med 1997;3:1280-4.
47. Sleat DE, Sohar I, Gin RM, Lobel P. Aminoglycoside-mediated suppression of nonsense mutations in late infantile neuronal ceroid lipofuscinosis. Eur J Paediatr Neurol 2001;5:Suppl A:57-62.
48. Keeling KM, Brooks DA, Hopwood JJ, Li P, Thompson JN, Bedwell DM. Gentamicin-mediated suppression of Hurler syndrome stop mutations restores a low level of alpha-L-iduronidase activity and reduces lysosomal glycosaminoglycan accumulation. Hum Mol Genet 2001;10:291-9.
49. Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. J Clin Invest 1999;104:375-81.
50. Dunant P, Walter MC, Karpati G, Lochmüller H. Gentamicin fails to increase dystrophin expression in dystrophin-deficient muscle. Muscle Nerve 2003;27:624-7.
51. Politano L, Nigro G, Nigro V, et al. Gentamicin administration in Duchenne patients with premature stop codon: preliminary results. Acta Myol 2003;22:15-21.
52. Wagner KR, Hamed S, Hadley DW, et al. Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. Ann Neurol 2001;49:706-11.
53. Wilschanski M, Yahav Y, Yaacov Y, et al. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and *CFTR* stop mutations. N Engl J Med 2003;349:1433-41.
54. Clancy JP, Rowe SM, Bebok Z, et al. No detectable improvements in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. Am J Respir Cell Mol Biol 2007;37:57-66.
55. Hirawat S, Welch EM, Elfring GL, et al. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. J Clin Pharmacol 2007;47:430-44.
56. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447: 87-91.
57. Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. Proc Natl Acad Sci U S A 2008;105:2064-9.
58. Kerem E, Hirawat S, Armoni S, et al. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. Lancet 2008; 372:719-27.
59. Leeds P, Peltz SW, Jacobson A, Culbertson MR. The product of the yeast UPF1 gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev 1991;5:2303-14.
60. Muhlrad D, Parker R. Recognition of yeast mRNAs as "nonsense containing" leads to both inhibition of mRNA translation and mRNA degradation: implications for the control of mRNA decapping. Mol Biol Cell 1999;10:3971-8.
61. Frischmeyer PA, van Hoof A, O'Donnell K, Guerrerio AL, Parker R, Dietz HC. An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. Science 2002;295: 2258-61.
62. Auld DS, Thorne N, Maguire WF, Inglese J. Mechanism of PTC124 activity in cell-based luciferase assays of nonsense codon suppression. Proc Natl Acad Sci U S A 2009;106:3585-90.
63. Vitiello L, Bassi N, Campagnolo P, et al. In vivo delivery of naked antisense oligos in aged mdx mice: analysis of dystrophin restoration in skeletal and cardiac muscle. Neuromuscul Disord 2008;18:597-605.
64. Lu QL, Mann CJ, Lou F, et al. Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse. Nat Med 2003;9:1009-14.
65. Mann CJ, Honeyman K, Cheng AJ, et al. Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse. Proc Natl Acad Sci U S A 2001;98:42-7.
66. van Deutekom JC, Janson AA, Ginjaar IB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007;357:2677-86.
67. Wu B, Li Y, Morcos PA, Doran TJ, Lu P, Lu QL. Octa-guanidine Morpholino restores dystrophin expression in cardiac and skeletal muscles and ameliorates pathology in dystrophic mdx mice. Mol Ther 2009;17:864-71.
68. Aartsma-Rus A, Janson AA, Kaman WE, et al. Antisense-induced multiexon skipping for Duchenne muscular dystrophy makes more sense. Am J Hum Genet 2004;74:83-92.
69. Aartsma-Rus A, Fokkema I, Verschuuren J, et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. Hum Mutat 2009;30:293-9.
70. Montes T, Tortajada A, Morgan BP, Rodríguez de Córdoba S, Harris CL. Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor B. Proc Natl Acad Sci U S A 2009;106:4366-71.
71. Klionsky DJ. Crohn's disease, autophagy, and the Paneth cell. N Engl J Med 2009;360:1785-6.
72. Yano T, Kurata S. An unexpected twist for autophagy in Crohn's disease. Nat Immunol 2009;10:134-6.