New Therapeutic Approaches to Mendelian Disorders

Harry C. Dietz, M.D.

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 and Leber’s congenital amaurosis caused by mutations in the gene encoding retinal pigment epithelium-specific protein of 65 kD (RPE65).

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. 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 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. 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. 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, modified targeting procedures, 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. 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). 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 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. 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 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-
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ERT denotes enzyme-replacement therapy, EU European Union, FDA Food and Drug Administration, MPS mucopolysaccharidosis, and SRT substrate-reduction therapy.

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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
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 activity and signaling. 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. Losartan also addressed manifestations outside the vascular system, including emphysema and myopathy. On this basis, the Pediatric Heart Network launched a clinical trial of losartan (ClinicalTrials.gov number, NCT00429364) in patients with Marfan’s syndrome. Although this trial has yet to be concluded, my colleagues and I 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.

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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),\textsuperscript{25,26} cutis laxa with aneurysm,\textsuperscript{27} the arterial tortuosity syndrome,\textsuperscript{28} and bicuspid aortic valve with aneurysm.\textsuperscript{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.\textsuperscript{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 non-receptor 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 (i.e., its Achilles’ heel).\textsuperscript{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.\textsuperscript{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,\textsuperscript{33} attention turned to the N-alkylated iminosugar N-(n-butyldedioxyojirimycin (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.\textsuperscript{34–37} Early results regarding miglustat therapy for neuronopathic manifestations of Gaucher’s disease have been less encouraging.\textsuperscript{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)deoxyojirimycin (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.\textsuperscript{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 Mogg44 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 colleagues45,46 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 dystrophy49,50 and in patients with either Duchenne’s muscular dystrophy51,52 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 dystrophy56 and cystic fibrosis57 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-
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 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.
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 and people with Duchenne’s muscular dystrophy. 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.

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 them70-72 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**

29. Gomez D, Al Haj Zen A, Borges LF, et al. Syndrome and non-syndromic aneu-
34. Pastores GM, Giraldo P, Chérin P, Mehta A. Goal-oriented therapy with mi-
term miglustat maintenance therapy in type 1 Gaucher disease: the ZAGAL pro-
38. Schiffmann R, Fitzgibbon EJ, Harris C, et al. Randomized, controlled trial of mi-
39. Fan JQ. A counterintuitive approach to treat enzyme deficiencies: use of en-
yme inhibitors for restoring mutant en-
40. Mu TW, Fowler DM, Kelly JW. Partial restoration of mutant enzyme homeosta-
sis in three distinct lysosomal storage disease cell lines by altering calcium ho-
41. Mu TW, Ong DS, Wang YJ, et al. Safety, tolerability, and pharmacoki-
netics of PTC124, a nonaminoglycoside bioavailable compound that promotes
ly bioavailable compound that promotes
amplification of mRNA translation in Duchenne patients with premature stop codon: pre-
42. Wagner KR, Hamed S, Hadley DW, et al. Gentamicin treatment of Duchenne and Becker muscular dystrophy due to non-
44. Clancy JP, Rowe SM, Bebok Z, et al. No detectable improvements in cystic fi-
brosis transmembrane conductance regu-
45. Hirawat S, Welch EM, Elfring GL, et al. Safety, tolerability, and pharmacoki-
netics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single-
and multiple-dose administration to healthy male and female adult volun-
87-91.
47. Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC124 is an or-
ally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. Proc
49. Leed P, Peltz SW, Jacobson A, Cul-
bertson MR. The product of the yeast UBF1 gene is required for rapid turnover of mRNA containing a premature transla-
50. Muhlead 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
51. Frischmeyer PA, van Hoof A, O’Donnell K, Guererro AL, Parker R, Dietz HC. An mRNA surveillance mecha-
nism that eliminates transcripts lacking termination codons. Science 2002;295:
2258-61.
52. Auld DS, Thorne N, Maguire WF, In-
glese J. Mechanism of PTC124 activity in cell-based luciferase assays of nonsense
codon suppression. Proc Natl Acad Sci
53. Vitiello L, Bassi N, Campagnolo P, et
al. In vivo delivery of naked antisense oligo-
gens in aged mdx mice: analysis of dystro-
phin restoration in skeletal and cardiac
muscle. Neuromuscular Disord 2008;18:597-
605.
54. Lu QL, Mann CJ, Lou F, et al. Func-
tional amounts of dystrophin produced by
skipping the mutated exon in the mdx
dystrophic mouse. Nat Med 2003;9:1009-
14.
56. van Deutekom JC, Janson AA, Ginjaar
IB, et al. Local dystrophin restoration with antisense oligonucleotide PR0051.
57. Wu B, Li Y, Morcos PA, Doran TJ, Lu P, Lu QL. Octa-guanidine Morpholino re-
stores dystrophin expression in cardiac
and skeletal muscles and ameliorates pa-
thology in dystrophic mdx mice. Mol Ther
2009;17864-71.
58. Aartsma-Rus A, Janson AA, Kaman
D, Sriram U, Hoen PA, et al. Antisense-induced multixon skipping for Duchenne muscular dystro-
phy makes more sense. Am J Hum Genet
59. Aartsma-Rus A, Fokkema I, Vers-
chuuren J, et al. Theoretic applicability of
antisense-mediated exon skipping for
60. Montes T, Tortajada A, Morgan BP,
Rodríguez de Córdoba S, Harris CL. Func-
tional basis of protection against age-
related macular degeneration conferred by a common polymorphism in comple-
ment factor B. Proc Natl Acad Sci U S A
2009;106:4366-71.
61. Klionsky DJ. Crohn’s disease, au-
62. Yano T, Kurata S. An unexpected twist
for autophagy in Crohn’s disease. Nat

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