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REVIEW

Drug Development for Rare Mitochondrial Disorders

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Abstract Currently, all treatment of mitochondrial disorders is performed with dietary supplements or by off-label use of drugs approved for other indications. The present challenge is translation of our collective knowledge of the molecular details underlying the pathophysiology of mitochondrial disorders into safe and effective therapies that are approved by the regulatory authorities. Molecular details permit precise diagnoses, but homogeneity is gained at the expense of limiting numbers of subjects for clinical trials and of small markets from which to recoup the considerable expense of drug discovery and development. The Food and Drug Administration recognizes that trial designs suitable for common diseases are often not feasible for rare disorders. They have developed a number of programs to facilitate development of novel therapies for such rare diseases, without compromise of regulatory standards. With advances in technology, including the use of biomarkers, replacement therapies and sophisticated trial designs, both biotechnology firms and, increasingly, large integrated pharmaceutical companies, are taking advantage of the opportunities in rare disorders. Precise molecular delineation of pathophysiology and of responsive patients has led to success rates with rare diseases that are significantly greater than those for common disorders. It appears likely, but not yet proven, that this may now be the case for rare mitochondrial disorders as well.

Keywords Drugs · Food and drug administration · Clinical trials · Rare diseases · Biomarkers · Mitochondrial

Introduction

There are two complementary approaches to the development of therapies for mitochondrial disease: a bottom-up approach that focuses on individual rare heritable disorders (Table 1) caused by mutations of a single gene (Table 2) or on top-down approach that addresses a more broadly defined, but prevalent, disorder, such as Parkinson disease or ischemic stroke (Table 3) in which mitochondrial dysfunction may be part of a more complex pathophysiology. The former offers homogeneity, but challenges in recruitment, clinical trial design, and marketing. The latter is more convenient, but also more risky. Many of the same candidate therapeutics have been used in both (Table 4) [1]. To date, neither approach has resulted in a Food and Drug Administration (FDA) approval for a mitochondrial therapy. The focus of this article will be on the former approach, drawing largely on practical lessons learned from non-mitochondrial monogenic and other rare disorders for which regulatory approval has already been achieved.

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Regulatory Status of Current Treatments of Mitochondrial Diseases

Therapies for mitochondrial diseases have been proposed and are being implemented, but none have gained FDA approval for marketing in this indication [2–7]. Therefore, all drugs currently being used for treatment of mitochondrial disorders are either unapproved, used off-label, or are dietary supplements. These are important distinctions.

Unapproved Drugs

Some older drugs continue to be marketed illegally in the USA without the required FDA approval or evidence of conformity to a monograph for making over-the-counter drugs. An *unapproved* drug is one that has not demonstrated that its manufacturing processes can reliably produce a product of expected identity, strength, quality, and purity, much less safety and efficacy for a given indication. Such drugs are considered a significant public health concern and are excluded from the *Orange Book*, the common name for the publication, *Approved Drug Products with Therapeutic Equivalence Evaluations*, a comprehensive listing of all drugs approved for

Table 1. Primary mitochondrial disorders resulting from a point mutation or a contiguous gene deletion

Ataxia and polyneuropathy, adult-onset [OMIM +516060]
Brain pseudoatrophy, reversible, valproate-induced, susceptibility to[OMIM +516070]
Bjornstad syndrome; BJS (pili torti and nerve deafness) [OMIM #262000]
Cardiomyopathy, apical hypertrophic, and neuropathy [OMIM +516070]
Cardiomyopathy, infantile hypertrophic [OMIM +516070]
Cardioencephalomyopathy, fatal infantile, due to cytochrome c oxidase deficiency [OMIM #604377]
Chronic progressive external ophthalmoplegia (CPEO)
Progressive external ophthalmoplegia, autosomal dominant (PEOA)
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal dominant*, *1*; [*OMIM # 157640*]
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal dominant*, *2*; *PEOA2* [*OMIM #609283*]
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal dominant*, *3*; *PEOA3* [*OMIM #609286*]
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal dominant*, *4*; *PEOA4* [*OMIM #610131*]
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal dominant*, *5*; *PEOA5* [*OMIM #613077*]
Progressive external ophthalmoplegia, autosomal recessive (PEOB)
Progressive external ophthalmoplegia with mitochondrial DNA deletions, *autosomal recessive*; *PEOB* [*OMIM # 258450*]
Progressive external ophthalmoplegia with a single mitochondrial DNA deletion, sporadic
Kearns-Sayre syndrome; *KSS* [*OMIM #530000*]
Friedreich ataxia I [OMIM #229300]
Gracile syndrome [OMIM #603358]
Leigh syndrome [OMIM #256000]
Leigh syndrome, French Canadian type; LSFC [OMIM #220111]
Mitochondrial DNA depletion syndromes
Mitochondrial DNA depletion syndrome 1 (*MNGIE type*); *MTDPS1* [*OMIM #603041*]
Mitochondrial DNA depletion syndrome 2 (*myopathic type*); *MTDPS2* [*OMIM #609560*]
Mitochondrial DNA depletion syndrome 3 (*hepatocerebral type*); *MTDPS3* [*OMIM #251880*]
Mitochondrial DNA depletion syndrome 4A (*Alpers type*); *MTDPS4A* [*OMIM #203700*]
Mitochondrial DNA depletion syndrome 4B (*MNGIE type*); *MTDPS4B* [*OMIM #613662*]
Mitochondrial DNA depletion syndrome 5 (*encephalomyopathic with methylmalonic aciduria*); *MTDPS5* [*OMIM #612073*]
Mitochondrial DNA depletion syndrome 6 (*hepatocerebral type*; *Navajo familial neurogenic arthropathy*); *MTDPS6* [*OMIM #256810*]
Mitochondrial DNA DEPLETION SYNDROME 7 (*hepatocerebral type*; *infantile spinocerebellar ataxia*, *with sensory neuropathy*; *OHAHA syndrome*); *MTDPS7* [*OMIM # 271245*]
Mitochondrial DNA depletion syndrome 8A (*encephalomyopathic type with renal tubulopathy*); *MTDPS8A* [*OMIM #612075*]
Mitochondrial DNA depletion syndrome 8B (*MNGIE type*) [*OMIM #612075*]
Mitochondrial DNA depletion syndrome 9 (*encephalomyopathic type with methylmalonic aciduria*; , *fatal infantile lactic acidosis*); *MTDPS9* [*OMIM #245400*]
Mitochondrial myopathy [OMIM #251900]
Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; MELAS [OMIM #540000]
Myoclonic epilepsy associated with ragged-red fibers; MERRF [OMIM #545000]
Pearson marrow-pancreas syndrome [OMIM # 557000]
Primary CoEnzyme Q10 Deficiencies
Primary CoEnzyme Q10 Deficiency Type 1 [*OMIM # 607426*]
Primary CoEnzyme Q10 Deficiency Type 2 [*OMIM # 614651*]
Primary CoEnzyme Q10 Deficiency Type 3 [*OMIM # 614652*]
Primary CoEnzyme Q10 Deficiency Type 4 [*OMIM # 612016*]
Primary CoEnzyme Q10 Deficiency Type 5 [*OMIM # 614654*]
Primary CoEnzyme Q10 Deficiency Type 6 [*OMIM # 614650*]
Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; SANDO [OMIM #607459]

McKusick et al. [54]

marketing in the USA [8]. In June 2006, the FDA announced a new drug safety initiative to remove unapproved drugs from the market, including a final guidance entitled "Marketed Unapproved Drugs—

Table 2. Genes in which mutation results in a mitochondrial disorder

RESPIRATORY COMPLEX I (NADH-CO Q REDUCTASE) DEFICIENCY
MITOCHONDRIAL COMPLEX I DEFICIENCY, NUCLEAR MUTATIONS [OMIM #252010]
—*C20ORF7*; *chromosome 20 open reading frame 7* [*OMIM *612360*]
—*NDUFA1*; *NADH-ubiquinone oxidoreductase 1 alpha subcomplex, 1* [*OMIM *300078*]
—*NDUFA11*; *NADH dehydrogenase 1 alpha subcomplex, 11* [*OMIM *612638*]
—*NDUFAF1*; *NADH dehydrogenase 1 alpha subcomplex, assembly factor 1* [*OMIM *606934*]
—*NDUFAF2*; *NADH dehydrogenase 1 alpha subcomplex, assembly factor 2* [*OMIM *609653*]
—*NDUFAF3*; *NADH dehydrogenase 1 alpha subcomplex, assembly factor 3* [*OMIM *612911*]
—*NDUFB3*; *NADH-ubiquinone oxidoreductase 1 beta subcomplex, 3* [*OMIM *603839*]
—*NDUFS1*; *NADH-ubiquinone oxidoreductase Fe-S protein 1* [*OMIM*157655*]
—*NDUFS2*; *NADH-ubiquinone oxidoreductase Fe-S protein 2* [*OMIM *602985*]
—*NDUFS4*; *NADH-ubiquinone oxidoreductase Fe-S protein 4* [*OMIM *602694*]
—*NDUFS6*; *NADH-ubiquinone oxidoreductase fe-s protein 6* [*OMIM *603848*]
—*NDUFV1*; *NADH-ubiquinone oxidoreductase flavoprotein 1* [*OMIM *161015*]
—*NDUFV2*; *NADH-ubiquinone oxidoreductase flavoprotein 2* [*OMIM *600532*]
—*NUBPL*; *nucleotide-binding protein-like protein* [*OMIM *613621*]
MT DNA COMPLEX I, MITOCHONDRIAL DNA MUTATIONS
—*Complex I, subunit ND1*; *MTND1* [*OMIM *516000*]
—*Complex I, subunit ND2*; *MTND2* [*OMIM*516001*]
—*Complex I, subunit ND3*; *MTND3* [*OMIM *516002*]
—*Complex I, subunit ND4*; *MTND4* [*OMIM *516003*]
—*Complex I, subunit ND4L*; *MTND4L* [*OMIM *516004*]
—*Complex I, subunit ND5*; *MTND5* [*OMIM *516005*]
—*Complex I, subunit ND6*; *MTND6* [*OMIM *516006*]
RESPIRATORY COMPLEX II (SUCCINATE DEHYDROGENASE) DEFICIENCY
—*Mitochondrial complex ii deficiency, nuclear mutations* [*OMIM #252011*]
RESPIRATORY COMPLEX III (CYTOCHROME B) DEFICIENCY
MITOCHONDRIAL COMPLEX III DEFICIENCY, NUCLEAR MUTATIONS [OMIM # 124000]
—*BCS1L*; *BCS1, S. cerevisiae, homolog-like* [*OMIM # 603647*]
—*TTC19*; *tetratricopeptide repeat domain 19* [*OMIM *613814*]
—*UQCRB*; *ubiquinol-cytochrome c reductase-binding protein* [*OMIM *191330*]
—*UQCRQ*; *ubiquinol-cytochrome c reductase, complex III subunit VII, 9.5-KD* [*OMIM *612080*]
MITOCHONDRIAL COMPLEX III DEFICIENCY, MITOCHONDRIAL MUTATIONS
—*Cytochrome b of complex III*; *MTCYB* [*OMIM *516020*]
RESPIRATORY COMPLEX IV (CYTOCHROME C OXIDASE) DEFICIENCY
MITOCHONDRIAL COMPLEX IV DEFICIENCY, NUCLEAR MUTATIONS [OMIM #220110]
—*Chromosome 2 open reading frame 64*; *C2ORF64* [*OMIM #613920*]
—*Chromosome 12 open reading frame 62*; *C12ORF62* [*OMIM #614478*]
—*Cytochrome c oxidase, subunit VIb, polypeptide 1*; *COX6B1* [*OMIM #124089*]
—*Cytochrome c oxidase assembly protein COX10*; *COX10* [*OMIM #602125*]
—*Cytochrome c oxidase assembly protein COX15*; *COX15* [*OMIM *603646*]
—*Cytochrome c oxidase assembly protein COX17*; *COX17* [*OMIM *604813*]
—*Fast kinase domain-containing protein 2*; *FASTKD2* [*OMIM #612322*]
—*Leucine-rich PPR motif-containing protein*; *LRPPRC* [*OMIM #607544*]
—*SCO1, S. cerevisiae, homolog of*; *SCO1* [*OMIM # 603644*]
—*SCO2, S. cerevisiae, homolog of*; *SCO2* [*OMIM # 604272*]
—*Surfeit 1*; *SURF1* [*OMIM #185620*]
—*TACO1* ; *Translational activator of mitochondrially encoded cytochrome c oxidase subunit I* [*OMIM # 612958*]
MITOCHONDRIAL MUTATIONS CAUSING COMPLEX IV DEFICIENCY
—*Complex IV, cytochrome c oxidase subunit I*; *MTCO1* [*OMIM *516030*]
—*Complex IV, cytochrome c oxidase subunit II*; *MTCO2* [*OMIM *516040*]
—*Cytochrome c oxidase, cytochrome c oxidase subunit III*; *MTCO3* [*OMIM *516050*]
—*Mitochondrial tRNA(ser)*; *MTTS1* [*OMIM #590080*]
—*Mitochondrial tRNA(leu)*; *MTTL1* [*OMIM #590050*]
RESPIRATORY COMPLEX V (ATP SYNTHASE) DEFICIENCY

Table 2. (continued)

COMPLEX V, NUCLEAR MUTATIONS
—*Mitochondrial complex V (ATP synthase) deficiency, nuclear type 1*; *MC5DN1* [*OMIM #604273*]
—*Mitochondrial complex V (ATP synthase) deficiency, nuclear type 2*; *MC5DN2* [*OMIM #614052*]
—*Mitochondrial complex V (ATP synthase) deficiency, nuclear type 3*; *MC5DN3* [*OMIM #614053*]
COMPLEX V, MITOCHONDRIAL MUTATIONS
—*ATP synthase 6*; *MTATP6* [*OMIM +516060*]
—*ATP synthase 8*; *MTATP8* [*OMIM +516070*]
NUCLEAR MUTATIONS CAUSING DEPLETION OF MITOCHONDRIAL DNA
—*DNA polymerase gamma*; *POLG* [*OMIM *174763*]
—*Deoxyguanosine kinase*; *DGUOK* [*OMIM *601465*]
—*MPV17, mouse, homolog of*; *MPV17* [*OMIM *137960*]
—*Ribonucleotide reductase, M2 B*; *RRM2B* [*OMIM *604712*]
—*Succinate-CoA ligase, alpha subunit*; *SUCLG1* [*OMIM *611224*]
—*Succinate-CoA ligase, ADP-forming, beta subunit*; *SUCLA2* [*OMIM *603921*]
—*Thymidine kinase, mitochondrial*; *TK2* [*OMIM *188250*]
—*Thymidine phosphorylase*: *TYMP* [*OMIM *131222*]
—*TWINKLE*; *chromosome 10 open reading frame 2*; *C10ORF2*; *TWINKY* [*OMIM *606075*]
OTHER NUCLEAR MUTATIONS
—*AARF domain-containing kinase 3*; *ADCK3* [*OMIM *606980*]
—*Adenine nucleotide translocator*; *solute carrier family 25 , member 4*; *SLC25A4* [*OMIM *103220*]
—*COQ2, S. Cerevisiae, homolog of*; *COQ2*; *Parahydroxybenzoate-Polyprenyltransferase, Mitochondrial* [*OMIM *609825*]
—*COQ6, S. Cerevisiae, homolog of*; *COQ6* [*OMIM *614647*]
—*COQ9, S. Cerevisiae, homolog of*; *COQ9* [*OMIM *612837*]
—*DNA polymerase gamma-2*; *POLG2* [*OMIM *604983*]
—*Frataxin* [*OMIM *606829*]
—*Prenyl diphosphate synthase, subunit 1*;*PDSS1* [*OMIM *607429*]
—*Prenyl diphosphate synthase, subunit 2*; *PDSS2* [*OMIM *610564*]
PYRUVATE DEHYDROGENASE COMPLEX
—*Pyruvate dehydrogenase, beta polypeptide*; *PDHB* [*OMIM *179060*]
—*Lipoic acid synthase*; *LIAS* [*OMIM *607031*]
—*Pyruvate dehydrogenase phosphatase catalytic subunit 1*; *PDP1* [*OMIM *605993*]
—*Pyruvate dehydrogenase complex, component X*; *PDHX* [*OMIM *608769*]
—*Pyruvate dehydrogenase, alpha polypeptide*; *PDHA1* [*OMIM *300502*]

McKusick et al. [54]

Compliance Policy Guide (CPG) [9]," outlining its enforcement policies aimed at efficiently and rationally bringing all such drugs into the approval process. In March 2011 the agency instructed manufacturers of some 500 prescription remedies that they would have 90 days to stop making these products and 180 days to end shipments. Drugs proposed for treatment of mitochondrial diseases have not been specifically cited in FDA publications.

Off-Label Use of an Approved Drug

In contrast, off-label use refers to the use of an approved product for any purpose or manner, other than described in its label. This includes treating with an approved product in either of three broad contexts: 1) for an indication other than the one for which the product was approved; 2) at a different dose or frequency other than that specified in the product's label; or 3) to treat a child when the product is only approved for the treatment of adults. An off-label use of a product can cease to be off-label if the product's maker submits a supplemental application and obtains FDA approval for the new use. The FDA encourages, but does not require, drug-makers to do this.

The FDA has the authority and responsibility to ensure that only treatments that are safe and effective for a specified indication can be introduced into interstate commerce. However, the FDA does not regulate the practice of medicine nor, for that matter, is it concerned about the relative efficacy of competing treatments. If the therapeutic product is manufactured legally and only promoted within the conditions specified by its label, any off-label use can be authorized by a

Table 3. Indications being investigated with therapeutics intended to remedy mitochondrial dysfunction

Disorder	Candidate therapeutic in treatment trials	Reference(s)
Aging	Coenzyme Q, latrepirdine	[95–97]
Alzheimer's disease	Coenzyme Q	[98–102]
Antiretroviral mitochondrial toxicity	Coenzyme Q	[103–107]
Cardiac ischemia	Coenzyme Q	[108]
Carnitine deficiency	Bezafibrate, carnitine	[109]
Collagen VI-related myopathies	Cyclosporine A	[110, 111]
Congenital lactic acidosis	Dichloroacetate	[46, 112]
Friedreich ataxia	Idebenone, coenzyme Q(10), and vitamin E; L-carnitine and creatine	[7, 17–24, 35, 36, 50, 113–119]
Parkinson disease	Coenzyme Q10 with creatine	[120–125]
Progressive supranuclear palsy	Coenzyme Q10	[126, 127]
Statin-related myopathy	Coenzyme Q10	[128–131]
Stroke	Transcranial near infrared laser	[132]

prescribing physician. In such a circumstance, the FDA attests to the reliable manufacture and distribution of a product of expected identity, strength, quality, and purity, but leaves the judgment about the safety and efficacy of such off-label use up to the discretion of the prescribing physician.

Dietary Supplements

A third regulatory category relevant to some proposed mitochondrial therapeutics is that of dietary supplements. For example, coenzyme Q10, creatine, vitamin E, and carnitine are considered *dietary supplements* rather than drugs provided that their labels only make so-called "structure-function" claims, such as maintenance of normal healthy structures or functions of the body. Dietary supplements are defined [10] as products taken by mouth that contain a "dietary ingredient" which may be "vitamins, minerals, herbs or other botanicals, amino acids, enzymes, organ tissues, glandulars (*sic*), and metabolites." These are regulated as foods, not as drugs. The FDA has published comprehensive regulations (*Current Good Manufacturing Practices*) for those who manufacture, package, or hold dietary supplements, thus ensuring their identity, purity, quality, strength, and composition. However, there are no provisions in the law for FDA to approve dietary supplements for safety or effectiveness for those introduced before 15 October 1994. Furthermore, manufacturers and distributors must record, investigate, and forward to the FDA any reports they receive of serious adverse events associated with their use. If the data from post-marketing surveillance warrants it, the FDA will post an alert [11]. No dietary supplement alerts have been posted for antioxidants or other dietary supplements proposed as therapies for mitochondrial disease.

The FDA's role depends on the nature of the label. If the label claims utility in the cure, mitigation, treatment, or prevention of a specific disease, the product would no longer be regulated as a food, but as a drug. To be marketed as drugs for the treatment of a mitochondrial disease, any compounds (including coenzyme Q10, creatine, vitamin E, or carnitine) will require approval of the FDA on the basis of demonstrated safety and efficacy using the same standards that are applicable to any new drug. Furthermore, the label must specify the disease for which it is indicated, as well as the method of use, including dosing regimen. For example, supplementation with coenzyme Q10 or ubiquinone would be considered the primary form of treatment in primary or secondary ubiquinone deficiencies [6, 7, 12–15], but not necessarily so in other mitochondriopathies where they are thought to exert a therapeutic effect as a supplemental electron transporter or antioxidant in the absence of a demonstrable deficiency. Similar considerations would apply to distinguishing treatment of primary carnitine deficiency [16] with oral carnitine to that of a mitochondriopathy, such as Friedreich ataxia [17].

Thus, although some purported treatments for mitochondrial disease are, and will continue to be, used legally, the present situation is far from optimal, for several reasons. First, even in these permitted uses, there has been no rigorous proof of clinical efficacy, although there have been reports of improvement not only in the primary deficiency states of coenzyme Q10 or carnitine, discussed above, but also in limited trials in other mitochondriopathies, as discussed elsewhere in this issue. Chief among these are reports of improvement in sonographic measures of cardiac hypertrophy of Friedreich cardiomyopathy [7, 18–22] after treatment with modest doses of idebenone. Even these findings require corroboration inasmuch as the improvements

Table 4. Orphan designations for mitochondrial disorders

Generic name	Designation date	Indication	Sponsor
Alpha-Tocopherol Quinone	03-28-2006	Inherited mitochondrial respiratory chain diseases	Penwest Pharmaceuticals Company
Alpha-Tocotrienol Quinone	10-21-2010	Inherited mitochondrial respiratory chain diseases.	Edison pharmaceuticals, Inc.
Cytochrome C, Flavin Mononucleotide And Thiamin Diphosphate	06-17-2011	Mitochondrial disorders	NBI Pharmaceuticals, Inc
5-[(E)-2-(4-Hydroxyphenyl)-Ethenyl] Benzene-1,3 Diol	03-13-2008	Treatment of MELAS syndrome	Sirtris Pharmaceuticals, Inc.
Idebenone	10-31-2006	Treatment of Leber's hereditary optic neuropathy	Santhera Pharmaceuticals Limited
Idebenone	05-22-2009	MELAS	Santhera Pharmaceuticals
Levocarnitine	04-07-1997	Zidovudine-induced mitochondrial myopathy.	Sigma-Tau Pharmaceuticals, Inc.
Modified Recombinant Mitochondrial Transcription Factor A (Tfam) Containing The Mitochondrial Transduction Domain. {OMIM 600438} 10q21.1	08-20-2012	Treatment of inherited mitochondrial respiratory chain disease	Gencia Corporation
Recombinant Thymidine Phosphorylase {OMIM *131222} Encapsulated With Autologous Erythrocytes	12-13-2010	Mitochondrial neurogastrointestinal encephalomyopathydue to thymidine phosphorylase deficiency	St. George's University of London
2',3',5'-Tri-O-Acetyluridine	01-13-2003 WITHDRAWN	Mitochondrial disease	Repligen Corporation
Ubiquinone	12-14-1999	Treatment of mitochondrial cytopathies.	Gel-Tec, Division of Tishcon Corp.

FDA Application Search Orphan Drug Designations and Approvals [53]

MELAS mitochondrial myopathy, encephalopathy, lactic acidosis with stroke-like episodes syndrome

were either observed in secondary biomarkers without demonstrable clinical correlates [18–21] or were the results of open-label trials that used historical rather than concurrent controls [20–22]. Furthermore, other studies failed to confirm these sonographic findings [23] and an independent study failed to provide clinical evidence of improved cardiac function [24], albeit with different dosing regimens and duration of treatment. Nevertheless, idebenone was licensed provisionally in Canada for treatment of Friedreich ataxia in July 2008 [25], but it has not been approved for such use either by the FDA or the European Medicines Agency.

Second, there has never been a powerful "home-run" effect with dietary supplements in any mitochondriopathy (other than in the aforementioned primary deficiency diseases) that would make such formal proof superfluous nor have there been careful attempts to optimize dosing regimens. Finally, limiting therapeutic options to dietary supplements and off-label use of drugs approved for other indications may be convenient, but it is suboptimal. We are failing to take full advantage of the remarkably rich pathophysiologic understanding of these disorders that has developed over recent decades [26–28]. Explorations of better options would best be undertaken in consultation with regulatory authorities.

Regulatory Hurdles for Rare Diseases

The significance of regulatory hurdles in the development of novel therapeutics for rare disorders, however, cannot be dismissed. This was a central theme at a recent conference convened by the Institute of Medicine [29]. The chairman of that conference, Professor Thomas Boat, wrote:

> Certain regulatory requirements undoubtedly lead pharmaceutical companies to put aside some drug development efforts that they might otherwise initiate or continue. Generating the evidence to support approval of a drug is costly and time-consuming for companies, and the potential always exists that pivotal clinical studies will not support safety or efficacy. In addition, the way the requirements are implemented may lead companies to put aside some potentially beneficial, innovative products, for example, if they expect or encounter difficulties in obtaining answers to questions or advice on trial design or if the review of their applications for approval of a product is slow or inconsistent across the FDA review divisions. When companies consider regulatory costs and uncertainties in addition to the expected size of the market,

candidate drugs that could meet the needs of small populations may be particularly vulnerable.

Regulatory Requirements for Standard Approvals

That having been said, the purpose of regulatory standards for all therapeutic agents is simple: the assurance of safety and efficacy. However, the technical details underlying regulatory approval can be dauntingly complex and often require the financial resources, as well as the expertise of companies who seek to recoup their investments by sales in large markets.

The regulatory requirements for approval fall into four broad categories: 1) Chemistry, Manufacturing and Control (CMC); 2) preclinical testing; 3) clinical development; and 4) post-marketing pharmacovigilance. Each of these is a discipline into and of itself. Furthermore, these regulations have changed over the years, most often after a public health crisis prompted Congress to issue new regulatory authority to the FDA or its predecessor organizations. A general understanding of this history helps explain the current priorities of regulators and the steps that must be taken by sponsors seeking approval.

CMC

The initial authority of regulators, then the Bureau of Chemistry in the Department of Agriculture, was strictly limited to enforcing standards of purity and identity: "What is in the bottle?" issues, without any regard to safety or efficacy. These CMC functions remain the bedrock of IND submissions, without which nothing else can happen. All large pharmaceutical companies have mastered the technical aspect of CMC, but these remain major stumbling blocks for small biotechs and academic investigators, who are more often focused on rationale and demonstration of efficacy. There is much available in the literature [30–34] on critical CMC issues: manufacturing, testing of stability, and purity both for traditional small molecule drugs, as well as manufacture and testing of protein therapeutics, isolation and maturation of stem cells, design of vectors, and delivery systems for advanced therapeutics based on nucleic acids. These all-important nuts and bolts details of manufacturing, storage, distribution, and quality control must be resolved precisely before any preclinical efficacy or safety testing begin. As development evolves from those in animals to initial and then pivotal studies in human subjects, the sponsor must demonstrate that the material that is used in all phases of development is not only what it is claimed to be, but also is pure, stable, and comparable wherever they have it manufactured. This must remain true not only in the smaller lots manufactured for early stages of preclinical testing or the larger lots used in later stages of development and in subsequent marketing. The critical questions are: 1) "What is the product?"; 2) "How is it manufactured?"; and 3) "Where is it manufactured?".

The sponsor must also describe the specifications of the analytical tools used to address questions of product identity, purity, and stability. For both historical and scientific reasons, regulations of small molecule drugs and of biologicals, though similar in intent, vary in detail. The origins of the present Center for Drug Evaluation and Research (CDER), the part of the FDA responsible for issuing New Drug Approvals (NDAs) for traditional small-molecule drugs, are in the Food Drug and Cosmetics Act of 1938, which built upon previous CMC requirements of drug purity and identity, to include requirements for safety. The Center for Biologics Evaluation and Research (CBER), the part of the present-day FDA that oversees the Biologic License Applications (BLAs) for traditional biologic products, such as vaccines and blood products, as well as advanced therapeutics, such as gene, cell, and tissue therapies, traces its origins back to a separate statute, the Public Health Service Act of 1902. More recently, CDER assumed responsibility for recombinant proteins from CBER, which continues to regulate all other classes of biologics. Scientific considerations also dictate different regulatory emphases for therapeutics that are small, analytically-defined molecules and for the much larger, complex biologics. For protein, as well as advanced, therapeutics, analytical tools are often inadequately sensitive for the provision of adequate assurances of these key attributes. Contamination by biologic agents (which cannot be eliminated by terminal sterilization of labile biologics), post-translational-modifications, denaturation, and aggregation may be biologically significant, even at a level that will elude detection by analytical techniques. Therefore, for such products the emphases are on bioassays and detailed descriptions of manufacture: "the process is the product."

Preclinical Testing

The requirements for product safety had its origins in the aforementioned Food Drug and Cosmetic Act of 1938, passed by Congress in response to an outbreak of more than 100 cases of renal failure and death resulting from the use of diethylene glycol as a solvent for the then wonder drug, sulfanilamide. Current safety regulations include the requirement for preclinical toxicological testing in healthy laboratory animals. This serves two purposes. First, is an estimate of how much can be given safely in subsequent clinical trials. The no observed adverse effect level is the highest dose that does not result in alterations of histology, function, growth, development, or life span. This dose informs selection of the first dose to be given in humans, after extrapolation by allometric scaling and reduction by a

safety factor of 10 or more, depending on the nature of the drug. Because many biologics do not demonstrate a no observed adverse effect level, selection of initial doses for human phase I studies are based on an alternative approach—minimal anticipated biologic effect level. The second reason for "preclinical tox" is prediction of the organ systems that are likely to be affected adversely, and, therefore, what special laboratory and clinical monitoring should be used in clinical trials. A separate reason for testing in animals is to obtain preliminary evidence of efficacy before testing in humans. However, it is only recently that meaningful animal models of some mitochondrial disorders have begun to emerge [6, 7].

Clinical Development

Phase 1 Phase 1 describes first-in-human studies that initiate clinical development. These can only commence after the FDA has approved an IND. Typically, these studies are performed in healthy adult male volunteers unless they are for cytotoxic therapy in oncology. In some trials for Mendelian inborn errors of metabolism, phase 1 studies are in patients, even children. The major purpose of a phase 1 study is to determine the maximum tolerated dose, an estimate based on cautious escalations (first in single-, then multiple-ascending dose studies). Dose escalation and trial design are different for those in which there is a high expectation of dose-limiting side effects (as for cytotoxic oncology drugs) and those for which the expectation is low (as in a gene-transfer protocol). For many biologicals, it is not possible to determine a maximum tolerated dose. This requires separate consideration of an optimal biological dose for subsequent studies.

The other purpose of phase 1 is determination of what the body does to the drug from pharmacokinetic data: measurements of absorption, distribution, metabolism, and excretion of the administered drug. Assessments of what the drug does to the body focus on safety issues and unwanted side effects. As will be discussed, clinical and standard laboratory measurements can sometimes be supplemented by special biomarkers designed to detect toxicity. In some metabolic diseases, phase I studies can also provide preliminary assessments of efficacy, either directly, or, most often, through the use of efficacy or dose-selection biomarkers. Observations of the dose-dependency of unwanted (and, where possible, desired) effects in the phase 1 studies guide dose selection in subsequent trials.

One of the few good examples of a phase 1 study for a mitochondrial therapeutic was conducted by the Neurogenetics Branch of the National Institute of Neurological Disorders and Stroke, National Institutes of Health in Bethesda, seeking to determine the safety, tolerability, and pharmacokinetics of increasing doses of idebenone [35]. The investigators performed an open-label, phase 1A dose-escalation trial in 78 patients with Friedreich ataxia , including adults, children, and adolescents, increasing in 10-mg/kg increments in each successive dose group to a maximum of 75 mg/kg. This was followed by an open-label, 1-month phase 1B trial in 15 patients at 60 mg/kg. No dose-limiting toxicity was observed and all observed adverse reactions were mild. In addition to clinical assessment of safety and tolerability, the investigators made pharmacokinetic measurements, including those of maximum drug concentration, time to maximum drug concentration, and half-life across age cohorts. This very well-performed study enabled effective design and execution of subsequent trials of efficacy for neurologic dysfunction in Friedreich ataxia, which appears to require higher doses than does the cardiomyopathy [36].

Phase 2 For common disorders, the next stage of drug development is phase 2, the purpose of which is to generate clinical data to help design phase 3 "pivotal" studies. Whereas the primary focus of phase 1 studies is safety, the focus of phase 2 is usually, and of phase 3 is always, on efficacy. However, safety monitoring continues throughout development in the progressively large cohorts of all phases, including phase 4 postmarketing pharmacovigilance.

It is interesting to note that marketed drugs were not legally required to be efficacious until passage of the Kefauver Harris Amendments of 1962, which were passed by Congress after multiple cases of phocomelia in infants whose mothers had taken thalidomide during pregnancy. The specific objective of phase 2 studies can vary from preliminary demonstration of efficacy (proof of concept), to investigations of dose and dosing regimens that focus on efficacy, as well as safety (dose-ranging), better characterization of a patient population, identification of an appropriate outcome measure for the pivotal studies, and/or further characterization of a safety issue. Typically, phase 2 studies are randomized, double-blinded placebo-controlled studies of 2 or more cohorts of individuals affected with the disorder for which the study drug is intended.

The majority of clinical trials for mitochondrial disorders listed in ClinicalTrials.gov [1], PubMed, and elsewhere in this issue correspond to typical phase 2 studies. There are hundreds. Several are worthy of specific mention in this review, even though they have not been submitted as components of regulatory dossiers. Tarnopolsky et al. tested creatine in 7 mitochondrial cytopathy patients using a randomized, crossover design, significantly improving several clinical measures of strength, as well as a biomarker, post-exercise lactate [37]. These observations have yet to be confirmed in a pivotal study in a mitochondrial or any other metabolic myopathy [38].

Studies in Leber hereditary optic atrophy (LHON) include two of idebenone by Klopstock et al. The first of these was the first randomized placebo-controlled treatment trial of any agent in LHON [39], as well as one of the first adequately powered, randomized controlled treatment trials for any mitochondrial DNA disease. Difficulties in recruiting acute cases led to inclusion of individuals with up to 5 years of visual loss. No statistically significant effect of treatment was seen with either the primary (best recovery in visual acuity) or secondary end points. In the second study with a similar population [40], treatment resulted in a demonstrable improvement in tritan, but not protan, color vision: scientifically interesting, but of uncertain clinical utility.

Small, uncontrolled trials in LHON have tested other vitamins and cofactors, including folic acid, coenzyme Q10, ascorbic acid, and cyanocobamalin, without success [41]. For that reason, the recent report of significant improvements in visual function in a small uncontrolled series of patients with LHON [42] after treatment with the novel agent EPI-743 [43] warrants follow-up with a larger, controlled series, ideally after a systematic phase I study of tolerability, safety, and pharmacokinetics. Similar promising results with small uncontrolled trials of EPI-743 warrant further systematic study in Leigh disease [44] and other mitochondriopathies [45].

The first double-blind, randomized, controlled trial of dichloroacetic acid in congenital lactic acidosis (a biochemically heterogeneous grouping of severe mitochondriopathies) was conducted by Stacpoole et al. [46]. This well-run study did not find that treatment improved any clinical outcomes, even though it favorably altered a biomarker—postprandial lactate. The same leaders have designed and implemented the first randomized controlled trial of coenzyme Q(10) in children with primary mitochondrial diseases [47], even though a previous randomized trial with this agent in another population of mitochondriopathies failed to show an effect [48].

Phase 3 If "proof of concept" is achieved in the phase 2 studies, the sponsor schedules an end-of phase 2 meeting [49] with the FDA to achieve agreement on trial design for "pivotal" phase 3 studies. Final approval of a drug typically requires a minimum of 2 "adequate and well-controlled (A&WC)" studies. The requirement of this minimum is based on the FDA's interpretation of the use of the plural word "studies" in the governing statute. This, of course, increases the statistical significance of claims of efficacy. The primary endpoints for these pivotal studies must be clinical assessments of how patients feel, function, or survive. The only exceptions in standard approvals are a small handful of validated "surrogate endpoints," such as blood pressure or cholesterol. As will be discussed, surrogate biomarkers that have not been fully validated can, however, be used as primary endpoints in pivotals for those disorders that qualify for "Accelerated Approval," albeit with the requirement of subsequent confirmation in studies using standard clinical measures as primary endpoints.

Typically, a phase 3 study is a randomized, double-blinded, concurrent placebo-controlled design. Exceptions are permissible, some more readily than others. Blinding and controls are critical. In "non-inferiority" designs there are positive, rather than placebo, controls. Such designs are problematic, and, in the case of mitochondrial diseases, not currently an option. More applicable, but still under-utilized, are randomized withdrawal designs. Designs especially suited to rare disorders are discussed in the following sections.

The challenges associated with design and conduct of a phase 3 study for mitochondriopathies are well described in a recent article by Peter Stacpoole [2]. Recent phase 3 studies in Friedreich ataxia, a relatively common disorder resulting from mutation of a single gene, have thus far failed to demonstrate amelioration of neurologic [50] or cardiac dysfunction [51].

Phase 4 After successful completion of 2 A&WC studies, the sponsor will receive FDA approval for marketing. FDA surveillance continues in order to ensure safety, appropriately-limited promotion, and compliant manufacturing. Safety monitoring is paramount in phase 4 post-approval studies. The 1500 or so patient exposures typically achieved before marketing approval for common disorders will only bring to light frequently occurring side effects and toxicities. Less frequent events may only be observed after tens or hundreds of thousands of exposures. The need for post-marketing surveillance is all the more germane when drugs are developed for rare disorders in which many fewer individuals will be exposed to the drug before marketing approval.

As with other FDA regulations, post-marketing regulations have evolved significantly over the years. The same Kefauver-Harris amendments of 1962 that first required demonstration of efficacy also expanded regulation of safety by requiring 1) disclosure of accurate information about side effects; 2) reporting of adverse reactions; and 3) provision of safe tolerances for unavoidably poisonous substances. After the Vioxx incident in 2004, post-marketing surveillance for safety signals evolved from passive systems to post-marketing commitments by sponsors for active pharmacovigilance for all drugs, including those that have become generic. In 2007 the Food and Drug Administration Amendments Act authorized an enforceable Risk Evaluation and Mitigation Strategy for any approved product whenever the agency considers it necessary to ensure that benefits outweigh risks [51].

Drug Development for Rare Disorders

These regulatory requirements and hurdles notwithstanding, many companies have found rare and even ultra-rare diseases to be attractive targets. All of these represent areas of unmet medical need—welcome exceptions to crowded markets already served by existing products. In the words of Thomas Boat [28], "rare diseases are not rare, at least in aggregate." Concerns about commercial viability have been dispelled by the experience of pioneers like Genzyme, Genentech, and a growing number of competitors. Indeed, in 2009 Wellman-Labadie and Zhou [52] reported that 18 drugs that had been approved solely for an orphan indication each had global sales of more than $1 billion.

But perhaps the greatest attraction of rare single gene disorders is the 15.3 % rate of regulatory approval [53], twice that for common disorders. Inappropriate choices of drug target and trial subjects are more frequent for multifactorial diseases than in disorders resulting from an identified gene mutation. The trade-off is between diagnostic certainty and convenience. Furthermore, detection of a modest therapeutic effect in a heterogeneous disorder often requires very large clinical trials.

For many common disorders, most therapies are small molecules, often antagonists that bind with high affinity to a macromolecular target chosen on the basis of a prevailing—but, more often than not, unproven—model of the disease. Similarly, just over 60 % of orphan designations for single gene disorders are for small molecule drugs [53]. Small molecules offer the obvious advantages of oral administration, ease of manufacturing and distribution. However, small molecules bring another level of complexity, with significant levels of off-target binding and resultant side effects and toxicities, even within the therapeutic range of most such drugs. More often than not, Paul Ehrlich's silver bullet is an aspiration rather than a reality. By way of contrast, off-target effects are unusual for protein therapeutics, which instead pose challenges of manufacture, instability at room temperature, limited distribution, immunogenicity, and the need for parenteral administration.

For many rare genetic disorders, the pharmacology is replacement, rather than receptor antagonism. There have been approvals for replacement with proteins (but not yet for advanced therapeutics, such as genes or modified cells) that provide the normal biochemical activity that was abrogated by mutation. Most of the replacement therapies that have gained approval from the FDA are for coagulopathies or non-mitochondrial metabolic disorders. In addition to the unparalleled advantage of being able to address the root cause of such usually recessive disorders, replacement of a protein that is normally endogenous also offers practical advantages in development. Clinical trials for replacement therapies can be more seamless and requirements for preclinical testing are often more modest. The effects of replacement therapy can be significant, even in small trials. Significant therapeutic effects have already been demonstrated for a handful of rare single-gene disorders by bone marrow transplantation (which is not regulated by the FDA if there is only minimal manipulation after harvesting) or by FDA-approved protein-replacement therapies. Adequate statistical power can be achieved in small, properly designed trials if effect size is large and variance is low.

Not all single-gene disorders, including those that cause mitochondrial diseases, are candidates for replacement therapy either with proteins, genes, or stem cells. Success with replacement therapies has been limited to a few recessive disorders, none of them mitochondrial. Replacement therapies would not be appropriate for genetically dominant disorders in which the mutant allele trumps the normal one by either a gain-of-function or another dominant-negative effect. Although provision of a normal gene product would not be useful, patients afflicted with some of these disorders might benefit from silencing of the mutant allele by RNA-interfering antisense oligonucleotides or similar technologies. However, there have only been three identified dominant mitochondrial disorders [54], all of them progressive external ophthalmoplegias (Table 2), associated with primary mitochondrial dysfunction.

Development and registration of either replacement or other therapies for recessive diseases provide other technical challenges, usually with delivery of macromolecules or cells to the target. Furthermore, many of those afflicted are young and medically fragile. Frequently, there is a need for novel endpoints for clinical trials, itself a source of regulatory complexity. These challenges notwithstanding, more than 400 clinical development programs for rare genetic disorders have been granted orphan disease designations [53], as will be discussed in the following. Seventy-five of these designates have subsequently been approved for marketing.

Design of Clinical Trials for Rare Diseases

Generally, FDA's clinical guidances apply to both drugs and biologics [55]. Clinical study designs for biologics are similar to those for small molecule drugs, but tend to be more cost effective. Typically, studies of biologics are designed to generate maximal efficacy data efficiently. As has been discussed, there are two overarching requirements for the clinical portion of any development program: 1) demonstration of efficacy in A&WC studies, (almost always using frequentist, Neyman-Pearson statistics with $p=0.05$, two tailed t test), as well as 2) an adequate safety database. Although these are the only absolute requirements, in standard development programs these requirements are fulfilled in the familiar paradigm of pre-approval testing in phases 1,

2, and 3 described earlier, with the 2 A&WC studies being phase 3, and the premarketing safety database being accumulated during all 3 phases. For common disorders, the typical size of cohorts for a phase 1 trial is 20–80; for phase 2 trials it is 100–300; for phase 3 trials it is 1000–3000 individuals. However, this is rarely optimal, or even achievable, for some rare diseases. "The concept that clinical drug development is comprised of four temporal phases, I through IV, is widely used. It is important to appreciate that this is a description not a set of requirements, and that for some drugs and development programs the typical sequence will not be appropriate or necessary" [56].

The number of patients that need to be studied is determined by 2 entirely separate considerations: the sample size required for statistical power to detect a treatment effect and also the entirely separate requirement for an adequate safety database. If, as in the case of some replacement therapies for autosomal recessive deficiency disorders the effect of treatment is dramatic, adequate statistical power can be achieved with small numbers of uniformly responsive study participants. Strong treatment effects and homogeneously responsive patients can dramatically reduce the sample size required for a given statistical power. This estimate is the primary responsibility of the sponsor because it can be calculated on the basis of his/her estimates of treatment effect and expected variability of response in the study population selected for the pivotal trials.

No such statistical calculation is possible to determine the required size of the safety database. This is a major issue for discussion with FDA at the end-of-phase 2 meeting. The FDA will not commit to total numbers at the pre-IND meeting. The starting point for discussions will likely be the recommendations of an ICH E9 guideline of 1500 participants being exposed to the drug at the doses to be recommended for marketing, including 300–600 participants being treated for 6 months and 100 participants treated for 1 year [57]. Such large cohorts can be recruited for common diseases in which mitochondrial dysfunction is thought to be a part of a more complex pathophysiology (such as Parkinson disease or stroke) (Table 3), but not for those rare disorders in which primary mitochondrial dysfunction is the direct result of a single mutation in a nuclear or mitochondrial gene (Table 1).

Clinical Development of Orphan Products to Date

Anne Pariser, Associate Director for Rare Diseases at the FDA, emphasizes that drugs developed for rare diseases are not held to a lower standard than those for common disorders [58]. Both must demonstrate substantial evidence of effectiveness/clinical benefit. This requires an adequate and well-controlled clinical study. Although the standards for the demonstration of efficacy are the same, of necessity they must be achieved more efficiently when the number of participants that can be enrolled in clinical trials is limited.

Mitsumoto et al. [59] conducted a review that compared design elements of 19 orphan drugs approved by the FDA for neurologic indications, with those of a contemporaneous group of 20 neurological drugs approved without orphan designation.

> All drugs for neurological diseases approved without an orphan indication included at least two randomized, double-blind, placebo-controlled trials. In comparison, 32 % of drugs with an orphan indication had at least two such trials and 74 % had at least one. Thirty-three pivotal trials were conducted for the 19 drugs approved with an orphan indication. Of the 33 trials, 11 (33 %) did not use a placebo control, 9 (27 %) were not double blind, and 4 (12 %) were not randomized. Drugs approved without an orphan indication had more pivotal trials per drug and a larger mean trial size (506 vs 164 trial participants).

In 2006 the 'Critical Path Opportunities Report' [60] identified two critical areas for improvement: "Our outreach efforts uncovered a remarkable consensus that the two most important areas for improving medical product development are biomarker development and streamlining clinical trials." Some measures have already been implemented. Further "streamlining" of clinical trials for rare disorders and implementation of biomarkers for these and others, are in progress, as will be discussed in the next two sections. It is important to be familiar with these precedents when proposing a development program in advance discussions with regulatory authorities.

Design of Small Clinical Trials

To achieve the required demonstration of efficacy with the limited number of potential participants available, clinical trials must be designed in such a way as to extract the maximal possible information from each subject [61]. This requires an unusually close working relationship between clinicians and statisticians. Such collaboration must begin at the earliest conception of the project and continue throughout the execution and subsequent analysis of the development program. A small clinical trial, as might be appropriate for evaluation of a treatment of a rare mitochondrial disease, is not just as smaller version of large clinical trial for a common disorder [62]. Among the designs of special interest to small clinical trials [63] are the variously named longitudinal, parallel group, crossover, add-on, n of 1, sequential, ranking, futility, and adaptive designs. Although discussion of each is beyond the scope of this review, it is important to note that some of these designs are only appropriate for certain mitochondrial disorders, but not for others.

For example, an n of 1 design might be appropriate for an episodic mitochondrial disorder, such as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, but not for a slowly progressive but highly heterogeneous disorder such as Kearns–Sayre syndrome.

Statistical Analysis of Small Clinical Trials

Statistical analysis of small clinical trials is a difficult, very specialized skill, familiar only to a relatively small group of practitioners [63]. This is because most workaday "frequentist" statistical methods used by clinical trialists and the FDA assume that the distribution of the mean of a sufficiently large number of independent random variables approximates a "normal" (i.e., *Gaussian*) distribution. This assumption allows the use of standard parametric methods [64]. These include *t* tests, analysis of variance, calculation of correlation coefficients, regression analysis, and the like.

The central limit theorem of probability algebra enables "large number statistics" through its demonstration that the mean of values from any distribution approaches the normal, provided that the variance of each is finite, and that there are enough of them. These criteria are fulfilled by almost anything that a drug developer would encounter in the course of doing a traditional large clinical trial. However, if *N* is not large, all this can break down pretty drastically. Altman and Bland [65] point out that using the alternative approach of trying to make estimates and calculations of confidence intervals by non-parametric "rank" methods require "extra assumptions which are almost as strong as those for *t* methods." Serious departures from a normal distribution, as can occur with a limited number of data points, require explicit modeling.

Parametric methods exist for many other distributions, but the clinician and the statistician need to work together to determine which is appropriate for a proposed small clinical trial. An even greater departure from routine regulatory procedure is the use of Bayesian methods [66], which have been used extensively for clinical trials in oncology and appear to be readily adaptable for application to some mitochondrial disorders. A Bayesian statistical analysis has already been used for a regulatory approval of a device by the Center for Devices and Radiological Health at FDA, but, as of this writing, not yet by CDER or CBER. A high degree of rigor is most certainly possible for the analysis of small samples [67], but it can be hard, unfamiliar territory to many statisticians. Many of these novel designs require extensive computer simulations, closed-form solutions not being practicable.

Several methods are available when it is critical to squeeze every last bit of precision from each participant in a target population that is very small [68]. Among them is the use of 1) more efficient continuous measures, 2) longitudinal measurements rather than just those at the end of the trial, 3) multiple measurements at each time-point to increase precision, and 4) optimal use of covariates. Such practices can help maximize extraction of useful information from each participant. However, it is important to always keep in mind the possibility that—contrary to earlier expectations—one might be able to find enough participants to execute a regular, properly powered, randomized, placebo-controlled parallel group design after all.

Alternatives are possible, but often not easy. There are well-known limitations to the use of historical controls, most importantly the evolution of the background standard of care. However, these are permissible under certain circumstances by the FDA. Although many of these statistical techniques would also work for large clinical trials, they are usually not worth the bother when one has the luxury of simply increasing the *N* to the point where the central limit theorem and the law of large numbers kick in. However, these techniques can be invaluable when one does not have that luxury. This is often the case for trials in precisely-defined mitochondrial disorders, for which the number of individuals for which the treatment is intended is limited severely. But, as with any departure from standard operating procedure, advance discussion with the FDA is mandatory.

Biomarkers

Ever since 2006, biomarkers have been advocated by the FDA Critical Path Initiative as a way of making development more efficient [69]. Despite an enormous uptake by industry, their utility in registration packages has, until recently, been far from clear. Clarity is beginning to emerge with the publication of two key documents: *Draft Guidance—Qualification Process for Drug Development* Tools [70] in October 2010, and *Guidance E16—Biomarkers Related to Drug or Biotechnology Product Development*: *Context*, *Structure*, *and Format of Qualification Submissions* in August 2011 [71]. The latter introduces the concept of "context of use." Unraveling the complexity requires recognition of several types of "contexts of use," each with distinct regulatory implications: biomarkers that allow 1) selection of responsive participants for clinical trial and for subsequent marketing, 2) optimization of dosing regimens, 3) preliminary assessment of efficacy, or 4) prediction of side effects or toxicity before they become clinically evident [72]. Each has different regulatory implications. The utility of each of these four general types of biomarkers varies according to the degree of validation, the business model of the company using them, and the terms under which regulatory approval is sought, as has been explored in a recent article in *Nature Reviews Drug Discovery* [73].

An excellent general overview of biomarkers for mitochondriopathies has recently been published by Suomalainen [74], and one focused on oxidative stress by Pandolfo [7]. Such biomarkers either identify the underlying mutation or one of its biochemical or physiological sequellae. The major metabolic consequence of a mitochondrial disorder is impairment of oxidative phosphorylation which results in 1) impairment of adenosine triphosphate production, 2) decreased oxygen consumption, and 3) compensatory overreliance on glycolytic metabolism. These are kinetic deficits, measurable as rates of oxygen consumption by polarography or enzymatic reactions measured *in vitro* in biopsied muscle or *in vivo* by examining the effects of graded exercise or recovery therefrom. Sometimes a measurement at a single point in time is sufficiently informative to preclude the necessity of estimating rates. If impairment exceeds a threshold for current metabolic demand (i.e., rate of production is exceeded by rate of utilization), there will be alterations in local concentrations of certain metabolites [75]: 1) decreased ratios of high-energy ATP and phosphocreatine to lower energy adenosine diphospate and adenosine monophospate (the phosphorylation potential) [76]; 2) the redox potential (which is related linearly to the phosphorylation potential and is also reflected in the ratio of reduced to oxidized glutathione); 3) increased concentrations and altered ratios of the end-products of glycolysis (reduced lactate, oxidized pyruvate, and/or amidated alanine). Certain of these local concentrations can only be measured by *in vitro* analysis of biopsied tissues, some can be quantified noninvasively by magnetic resonance spectroscopy. Finally, some of these local alterations can be of sufficient magnitude to be reflected in systemic concentrations in blood or spinal fluid: notably, lactate/pyruvate/alanine.

When measurements of such quantities fall outside the range of values observed in normal individuals in similar circumstances, they can be considered diagnostics (or, for purposes of the discussion that follows, "patient selection biomarkers"). Alternatively, if these quantities revert from an abnormal baseline toward normal after treatment, they can be used as "efficacy biomarkers" or, alternatively, as "dose-selection biomarkers", described later. However, the utility of these mitochondrial biomarkers depends on careful consideration of the severity and tissue distribution of the impairment (important not only in variably heteroplasmic mitochondrially inherited disorders [77], but also poorly understood selective tissue involvement in nuclear-encoded [75], as well as homoplasmic disorders, such as LHON) and the relative rates of synthesis to that of consumption at the time of sampling, which can vary dramatically with fed/fasting state and exertion. As a general rule, the more subtle the defect, the more rigorously controlled the conditions of sampling must be if there is to be meaningful use of any of these biomarkers.

To these commonly used measurements may be added three promising novel biomarkers. Fibroblast growth factor 21 is a molecule that normally serves as a signal of starvation [77], but blood levels of which have been found elevated in mitochondrial disorders that affect skeletal muscle, even in fed states [78, 79]. Citrullinemia and reduced levels of intracellular glutathione in peripheral blood leukocytes may be more reliable measures of oxidative stress than are direct measures of highly-reactive and hence short-lived reactive oxygen or nitrogen species [80]. Measurement by single photon emission computerized tomographic scanning of brain uptake of technetium-99 m hexamethylpropyleneamine oxime, Tc99m-exametazime, Ceretec™, may be a useful imaging biomarker of reduced glutathione and reduced protein thiols in patients with a variety of mitochondrial encephalopathies [81].

Efficacy Biomarkers

The use of biomarkers that has attracted the greatest attention both in industry and the academic press has been as an early sign of efficacy. The vast majority of proposed efficacy biomarkers are not recognized to be "a *surrogate marker* (that) can be defined as a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy" (my emphasis) [82]. None of the biomarkers for mitochondrial disease discussed earlier have been validated to the level necessary to allow them to serve as a primary endpoint in a pivotal registration trial. Such *non-surrogate efficacy biomarkers* have no direct regulatory consequences in standard approval protocols, but they can be useful for internal decisions, such as portfolio prioritization. However, use of efficacy biomarkers that are not sufficiently accurate can even be problematic for certain internal go-no-go development decisions [73].

This is not the case for programs developing treatments under the Accelerated Approval process. This most significant distinction allows the use of an efficacy biomarker as a primary endpoint in pivotal trials, even if it had not been validated sufficiently for use in a traditional approval process. "An unvalidated surrogate biomarker cannot substitute for a clinically meaningful endpoint in pivotal registration trials except under Subpart H of 21 CFR 314.500 (Code of Federal Regulations) known as the Accelerated Approval provisions" [83, 84]. A similar statutory exemption (Subpart E) exists for some biologics [85].

For development programs that do not qualify for Accelerated Approval, many of these measures may be used as *dose-selection biomarkers*, as described in the following.

Toxicity Biomarkers

Biomarkers predicting side effects or frank toxicity are as difficult to validate to a standard that merits approval as a surrogate in a traditional registration procedure as are biomarkers of efficacy [73]. Nevertheless, some biomarkers of toxicity can help in the selection of a therapeutic candidate from multiple possible compounds by filtering out those for which toxicity problems seem more likely. Such biomarkers are increasingly being developed and applied.

Patient-selection Biomarkers

It is this category of biomarker that potentially offers the greatest utility for the development of those mitochondrial therapies that target specific defects. Some patients may respond to certain therapies, whereas others will not. Patient-selection biomarkers, when available, identify which is which. These are essential for treatment of rare genetic disorders, not only for confirmation of the diagnosis so as to permit enrollment only of eligible participants, but also, in some instances, for precise molecular definition of the defect in a given patient. For some proposed treatments such detail may be critical, whereas for others meticulous diagnostic precision may be irrelevant. For example, generic antioxidant treatment may reasonably be expected to treat a commonly occurring deleterious consequence of any of a large number of different mutations, though even some of these may need to be tailored for specific disorders [7]. However, some therapies would only be useful in very specific circumstances. For example, it may prove useful to reduce the toxic accumulations of the nucleosides thymidine and deoxyuridine that underlie instability of mitochondrial DNA in mitochondrial neurogastrointestinal encephalopathy, which is caused by mutations of thymidine phosphorylase [12]. However, this therapeutic approach is unlikely to be successful in other mitochondriopathies. Therefore, for highly targeted therapies, patient selection biomarkers based on the causative mutation may prove indispensable, just as they have already proven themselves in oncology.

Of the 11 orphan designations for mitochondrial disease, only 1 is defined in precise molecular terms (Table 4) [53]. There is no imperative from regulatory authorities on either side of the Atlantic for extensive validation of patient-selection biomarkers nor do they require designation as surrogates. Trial sponsors may define entry criteria as narrowly as they like. However, they do so with the responsibility to provide a companion diagnostic, which itself must meet regulatory standards for reliability.

This class of patient-selection biomarkers was pioneered by sponsors developing targeted non-cytotoxic therapies in oncology, which claim, by far, the largest number of orphan designations and approvals [53]. It started with Her 2/neu, a biomarker that identifies those breast cancers that will respond to Herceptin. This marked a turning point for acceptance of biomarkers by the pharmaceutical industry. Over the ensuing decade it has been learned that only about 10 % of cancer patients respond to any given targeted (i.e., non-cytotoxic) pharmacotherapy. Patients who will respond to certain other targeted therapies can be identified by one of about a dozen such biomarkers that have been reported to date [86]. FDA-approved labels require testing for some of these biomarkers before individual prescriptions for certain treatments. Biomarkers for others are only recommended or mentioned for information.

Molecular diagnosis of genetic mitochondrial disorders may allow similar, or even greater, precision in the identification of potentially responsive patients than these patient-selection biomarkers for oncology, where frequent somatic mutations may diminish response to previously effective targeted therapy. Although they have not been used extensively in drug development for mitochondrial disorders (Table 3), identified mutations have the potential of doing so (Table 2). Such a focus on an ultra-rare disease would, undoubtedly, make patient recruitment and size of the eventual market more problematic, but it may increase the likelihood of therapeutic success. In oncology, initial concerns about limitation of market share and the burden imposed by required testing with biomarkers have been more than offset by the benefits of efficient clinical trials, faster market penetration, as well as by ethical considerations.

Dose-selection Biomarkers

FDA senior staff repeatedly cite dosing as a root cause of many regulatory problems. A highly placed official (who asked to remain anonymous) remarked, “I am amazed at how often sponsors miscalculate dosage: both too high and too low.” Dose-selection biomarkers do not require recognition as surrogates by regulatory agencies, inasmuch as sponsors are given considerable latitude in the choice of dosing regimens, bounded only by the need for acceptable safety margins defined by preclinical and phase 1 studies. As is the case for patient-selection biomarkers described earlier, the use of dose-selection biomarkers are not problematic for regulators. In the words of Rusty Katz of the FDA, “The use … of biomarkers in early phases of drug development is, from a regulatory perspective, noncontroversial… For example…markers to determine presumed effective doses … these uses of biomarkers … are encouraged” [87].

Such biomarkers are typically applied early in development to a small number of volunteers, rather than the entire cohort enrolled in subsequent phase 2 and 3 trials. However, in the case of heteroplasmic mitochondrial DNA disorders,

it may prove useful to apply dose-selection markers to each participant enrolled in a pivotal trial of certain proposed therapeutic agents, as a means of optimizing a dosing regimen. Receptor-occupancy biomarkers, such as those afforded by ligand-displacement positron emission tomography or single photon emission computerized tomographic scanning in neuropsychiatric diseases to quantify the extent of binding of antagonist drugs, are the most direct and conceptually most attractive. However, such biomarkers would not be applicable for most mitochondrial therapies, for any of a number of reasons. It is more likely that a less direct pharmacodynamic biomarker, such as a measurement of brain phosphorylation potential or lactate level by magnetic resonance spectroscopy, before and after dosing, to be useful [76]. For example, in Phase 1 studies doses of the putative therapeutic agent could be titrated upward until the biomarker normalized. Then, this dose could be taken forward into pivotal trials using standard clinical measures as primary endpoints.

Regulatory Resources for Rare Diseases

It is the stated policy of the FDA that the standards for safety and efficacy applicable to treatments for rare diseases are the same as those for common ones. However, the agency has taken many steps to reduce regulatory burdens that may be hindering development of innovative therapies, particularly for serious disorders for which there are as yet no effective therapies, as well as for rare disorders with a limited patient base for the conduct of clinical trials and subsequent marketing of successfully approved products.

Orphan Drug Status

The Orphan Drug Act of 1983 was amended in 1984 to specify applicability to disorders with prevalence in the USA of < 200,000, without the earlier requirement that applicants demonstrate inability to recoup the costs of development from projected sales. Of 2649 orphan-designated products [53], 486 have been for single gene disorders and only 11 have been for mitochondrial disorders (Table 4), when narrowly defined as primary dysfunction of the electron transport chain (Table 4): 3 for mitochondrially-inherited disorders (LHON or mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); 1 for an autosomal recessive Mendelian disorder (mitochondrial neurogastrointestinal encephalopathy resulting from mutation of the nuclear-encoded thymidine phosphorylase gene); 1 for drug-induced mutations of the electron transport chain (by zidovudine); the remaining 6 apparently intended for treatment of any of a number of either mitochondrially-encoded, Mendelian, or X-linked heritable disorders of the electron transport chain or upstream enzymes, such as pyruvate dehydrogenase.

There are significant advantages to obtaining an orphan designation: 7 years' protection from market competition for approved orphan drugs (market exclusivity); grants to support product development; tax credits for certain costs associated with clinical trials on orphan drugs; waiver of application fees; and advice to product developers on the design of studies of safety and effectiveness to meet regulatory standards . However, the requirements for demonstration of safety and efficacy are not relaxed for orphan drugs. Nevertheless, 407 orphan-designated products have already received marketing approval at the time of writing this (September 2012) [53, 88, 89]. Half of these approvals came within 4 years of designation. The most common population size was fewer than 10,000 patients. Seventy-five of these approvals have been for rare, single-gene Mendelian, or X-linked disorders. None were for mitochondrial diseases.

Other Regulatory Resources That May be Applicable to Some Rare Mitochondrial Diseases

On 9 July 2012, President Obama signed into law the Food and Drug Administration Safety and Innovation Act (FDASIA) [90], which includes several sections that may facilitate development and registration of certain therapies, including some for rare mitochondrial disorders. These include sections under Title IX: Drug Approval and Patient Access, including provisions for consultation with external experts on rare diseases (section 903), for grants and contracts for the development of orphan drugs (section 906), and for the rare pediatric disease Priority Review voucher incentive program (section 908).

Two sections of Title IX merit detailed discussion. Section 901 codifies and clarifies the relationship between the pre-existing Accelerated Approval and Fast Track programs that had initially been spurred by the human immunodeficiency virus–acquired immune deficiency syndrome crisis in order to speed development and approval of innovative and effective drugs for the treatment of serious, life-threatening diseases. In addition, section 901 also makes additional provisions to the Priority Review Program [83]. In contrast, section 902 lays the groundwork for a brand new, but not yet completely defined, "Breakthrough Therapies" program. Several mitochondrial disorders may be eligible for one or the other of these programs, as will be discussed.

Fast Track Designation

Fast Track designation [83] was instituted to expedite the development and the review of drugs that will not only treat serious diseases, but will also fill an unmet medical need,

irrespective of the prevalence of the disease. Programs that receive Fast Track designation are eligible for Accelerated Approval and most will also be considered appropriate for Priority Review. As will be discussed, these complementary programs are administered separately and have distinct, well-specified eligibility criteria. Each requires an individual application from the sponsor according to a specified format and protocol. Examples of serious diseases eligible for Fast Track designation include acquired immune deficiency syndrome, Alzheimer's disease, heart failure, cancer, epilepsy, depression, and diabetes. Several mitochondrial diseases are likely to qualify, but there is no published example. The second requirement of unmet medical need is defined as provision of a therapy where none exists or of one that potentially may be superior to existing therapy by virtue of either superior effectiveness, the avoidance of serious side effects or a decrease in a clinically significant toxicity of an accepted treatment. Successful applicants for Fast Track designation are granted more ready access to the FDA including "rolling review," the prerogative of submission of sections of a NDA for review by FDA as they are completed, rather than waiting for review after completion of an entire application.

Although these benefits may appear modest, they could be invaluable, especially in a novel area, such as the development of mitochondrial therapies.

Accelerated Approval Based on Surrogate Efficacy Biomarkers

There is an important exception to the extremely strict validation requirements for biomarkers to be used as primary endpoints in pivotal studies. Subpart H 21 CFR 314.510[46] states that the FDA may grant marketing approval for a new drug product on the basis of adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate endpoint that is *reasonably likely*, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit or on the basis of an effect on a clinical endpoint other than survival or irreversible mortality [84]. Similar language is provided for biologics in Subpart E (§601.40) [85]. Definitions provided by the Code of Federal Regulation define eligibility. "Life-threatening" diseases are defined as 1) diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted, and as 2) diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival. "Severely debilitating" diseases are defined in 314.81(b) as diseases or conditions that cause major irreversible morbidity.

In both programs there is a requirement that the applicant study the drug further after market approval to verify and describe its clinical benefit where there is uncertainty, either of the surrogate to clinical benefit or of the observed clinical benefit to ultimate outcome. Sponsors applying for these programs usually have planning for phase 4 post-marketing studies well underway at the time of application. These must be adequate, well-controlled, and carried out with due diligence. Under Subpart H, the FDA may withdraw approval, following a hearing if the post-marketing clinical study fails to verify clinical benefit, the applicant fails to perform the required post-marketing study with due diligence, the promotional materials are false or misleading, or there is other evidence demonstrating that the drug product is not shown to be safe or effective under its conditions of use [84].

The Government Accountability Office tabulated 64 NDAs and BLAs that had been approved on the basis of surrogate biomarkers as of 20 November 2008 [91]. This was not uniform across all reviewing divisions. Indeed, Rusty Katz of the FDA had cautioned earlier that "It is worth noting that the FDA does not have a monolithic position on the approval of drugs under the Accelerated Approval regulations" [87]. Each division has its own policy.

Priority Review

Unlike the requirements for Accelerated Approval or Fast Track, Priority Review status [83] can also apply to drugs for less serious illnesses. This program provides extra FDA resources to the review of selected programs, with the goal of achieving an NDA (for drugs or genetically engineered proteins) or BLA (for other biologics) review within 6 months of submission instead of the standard 10 months. It is intended for development programs that provide evidence of increased effectiveness, elimination or substantial reduction of treatment-limiting drug reactions, documented enhancement of patient compliance, and/or evidence of safety and effectiveness in a new subpopulation, such as children. Section 908 in the FDASIA of 2012 establishes a "Priority Review voucher" for sponsors of a rare pediatric disease product application that entitles the holder to a Priority Review of a single human drug application [90].

Special Protocol Assessments

Special protocol assessments permit FDA pre-agreement with sponsors on design and size of planned clinical trials [92]. This program is available for all diseases, not just serious disorders. Harking back to 2002, this program enables rapid FDA evaluation of certain protocols to assess if they are adequate to meet scientific and regulatory guidelines. One of the 3 types of protocols eligible for such assessment are protocols for phase 3 trials whose data will form the primary basis for an efficacy claim. The eligible

clinical protocols can relate to efficacy claims that will be part of an original NDA or BLA, or that will be part of an efficacy supplement to an NDA or BLA that has already been approved.

Breakthrough Therapies

Unlike the previously-described programs, Breakthrough Therapies only became effective in October 2012 and will not be fully defined until the issuance of a Guidance—18 months after the July 2012 passage of FDASIA [90]. The intent of this program appears to be provision of an even more rapid route to approval than is allowed by Fast Track designation, perhaps from a process of "progressive approval" that would allow marketing to begin before typical pivotal studies are completed. This is intended for programs that provide preliminary evidence of a dramatic improvement over existing therapies, such as "a hazard ratio of .5 or an 80 % improvement in overall survival rather than meager improvement in overall survival," in the words of Richard Pazdur of the FDA. Although the advocates for this program were concerned primarily with oncology, this program would appear to be available for all indications, including rare mitochondrial disorders for which there is preliminary clinical evidence of a "home run effect [93]."

Pitfalls to be Avoided

Although there have been 11 orphan product designations for rare mitochondrial disorders, at the time of writing in September 2012, there have, as yet, been no successful precedents of regulatory approval for a rare mitochondrial disorder. However, there are many examples of drug development for other single gene disorders that may prove useful as exemplars. There are presently 478 FDA designations of Orphan Status for proposed treatments of other single-gene Mendelian or X-linked disorders, with a skewed distribution among 122 disorders, 8 with more than 10 designations each. In descending order, designations were awarded to medium-to-small cap biotechnology firms (322), large pharmaceutical houses (117), unaffiliated individuals (22), academic institutions (10), disease-related charities (6), and a single governmental agency. The overall-rate of marketing approval for single-gene disorders has been 15.7 %, nearly identical to the 15.3 % approval rate for all 2658 designated orphans, the majority for oncology. However, the approval rate for Mendelians varies significantly with therapeutic class [small molecule (9.4 %) or proteins (31.1 %)] and the nature of the sponsor. Among the 234 biologics designated for single-gene disorders, all 52 approvals have been for proteins, but none for advanced therapeutics (cells, genes, inhibitory oligonucleotides) even though advanced therapeutics have received 67 orphan designations for single-gene disorders. One advanced therapeutic, a cell therapy, did receive marketing approval in 2010 for oncology. Single-gene disease orphans sponsored by large pharmaceutical companies achieved an overall approval of 34.1 % versus 10.6 % for those sponsored by smaller biotechnology firms. The approval advantage of large integrated pharmaceutical companies over all other, smaller sponsors spanned therapeutic classes: proteins 47.1 % versus 20.2 %, small molecules 19.0 % versus 7.4 %. These highly discrepant approval rates correlate both with available resources, as well as with experience.

In our rather broad, but admittedly biased, experience as consultants advising development and regulatory strategies for therapies intended for a wide variety of indications, my colleagues and I have found that the most frequent difficulties encountered by small sponsors of therapeutics for rare single gene disorders result from either inability to demonstrate consistent manufacturing and safety, suboptimal clinical trial design, inexperience with developmental and regulatory procedures, and/or suboptimal interactions with the FDA. This appears to be the case even when small sponsors might have greater expertise in the pathophysiology and clinical management of a rare disorder than do investigators in large companies. Although the focus of small sponsors is frequently centered on proof of concept, equal attention needs to be afforded to CMC, safety, dose selection, and design of pivotal trials. These are similar to conclusions of a recent study by Harald Heemstra et al. from the Medicine Evaluations Board in the Netherlands [94] who found that the most frequent reasons for non-approval for marketing of candidates with Orphan Designation were "clinical trial design, the level of experience of the sponsor and the level of interaction with the FDA."

At a conference that the Institute of Medicine convened in October 2010 to examine the development of therapies for rare diseases, Dr Anne Pariser described recurrent problem areas encountered by her agency in applications for approval for Orphan Diseases [29]:

- incomplete NDA or BLA applications;
- toxicology studies not completed on a timely basis;
- inadequate characterization of the chemical compound;
- lack of advance communication with FDA about adequacy of plans for clinical trials;
- lack of natural history studies to characterize the disease process, including variability in disease severity, symptom stability, and outcomes;
- poor use of early-phase safety and dosing studies to inform phase III or pivotal studies;
- inadequate trial design, including lack of formal protocols, poorly defined questions, inadequate control

groups, and lack of validated biomarkers and appropriate surrogate measures.

These are all tractable problems, avoidable with proper planning.

Conclusion

As is evident from other articles in this issue of *Neurotherapeutics*, development of effective therapies for rare mitochondrial disorders is very much needed. Despite significant technical and logistical challenges, these disorders also offer scientific, clinical, and commercial opportunities. These can only be realized fully by strict adherence to regulatory standards, which can be complex. Although there are no direct precedents for gaining regulatory approval for treatments of rare mitochondrial diseases, a wide variety of successful approvals for other orphan disorders, particularly those for other single-gene disorders, suggest that such approval can be attainable.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article

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