INVITED REVIEW

Progress toward improved therapies for inborn errors of metabolism

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Abstract

Because of their prevalence, severity and lack of effective treatments, inborn errors of metabolism need novel and more effective therapeutic approaches. The opportunity for an early treatment coming from expanded newborn screening has made this need even more urgent. To meet this demand, a growing number of novel treatments are entering in the phase of clinical development. Strategies to overcome the detrimental consequences of the enzyme deficiencies responsible for inborn errors of metabolism have been focused on multiple fronts at the levels of the gene, RNA, protein and whole cell. These strategies have been accomplished using a wide spectrum of approaches ranging from small molecules to enzyme replacement therapy, cell and gene therapy. The applications of new technologies in the field of inborn errors of metabolism, such as genome editing, RNA interference and cell reprogramming, along with progress in pre-existing strategies, such as gene therapy or cell transplantation, have tremendous potential for clinical translation.

Introduction

Since its introduction by Garrod in 1908 (1), the concept of inborn error of metabolism has significantly expanded to include over 300 disorders (2). These diseases are due to defects in a protein (more often an enzyme or a transporter) that results in accumulation of intermediary metabolites that cannot be further processed. Although individually rare, their collective incidence is estimated to be 1 in 4000 newborns (3,4). Because of their prevalence and severity, significant efforts have been made over the last few decades to develop novel and more effective strategies to treat the disease complications, prolong survival and improve quality of life. Nevertheless, effective therapies are still lacking for most of these disorders. The implementation of expanded newborn screening based on tandem-mass spectrometry has allowed early diagnosis for several metabolic diseases (5,6) and offers the unprecedented opportunity for an early treatment thus making the development of more effective therapies more urgent.

Current Treatments

The pathogenesis of inborn errors of metabolism can be explained by (i) deficiency of essential product/enzyme, (ii) deleterious systemic effects of circulating toxic metabolites or (iii) activation of abnormal alternative metabolism. Based on these mechanisms, the therapy of inborn errors of metabolism is traditionally aimed at ameliorating the disease by restriction of upstream nutrients to prevent intoxication, supplementation of downstream nutrients to prevent secondary deficiency, stimulation of alternative routes for disposal of precursor metabolites or replacing the defecting enzyme by intravenous protein infusions (7) (Fig. 1).

Dietary manipulations typically aim at reducing the intake of toxic precursors or at providing the deficient products. Vitamins that are enzyme cofactors are given because some disorders are caused by defects in the synthesis, transport or metabolism of cofactors, as in biotinidase deficiency or cobalamin disorders. Moreover, cofactor administration can also increase the activity

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Figure 1. Strategies for therapies of inborn errors of metabolism.

of the defective enzyme by increasing at least in part its stability. Tetrahydrobiopterin (BH4), the cofactor of phenylalanine hydroxylase is used for defects of BH4 synthesis and regeneration but also for phenylketonuria because it enhances enzyme residual activity and thus, it increases phenylalanine tolerance (8). Similarly, riboflavin has positive effects on multiple acyl-CoA deficiency (9).

Enzyme replacement therapy (ERT) based on the administration of the deficient enzyme has been developed for a growing number of lysosomal storage disorders (10). ERT is based on the ability of most cells to uptake the exogenous enzyme through the mannose or mannose-6-phosphate receptors present on their cell surface and deliver it to lysosomes (11). Based on the extensive clinical experience now available, despite measurable clinical benefits, it has become clear that ERT has important limitations related to tissues that are more refractory to treatment and to immune reactions (12). Targeting of the enzyme to tissues such as bone, cartilage or brain is limited. Over the last few years, efforts to target the brain have indeed been focused on intrathecal rather than intravenous enzyme delivery. Biodistribution to more resistant tissues has potential to be improved by addition of tissue-specific targeting peptides. Tissue-non-specific isozyme of alkaline phosphatase has been coupled to a deca-aspartate motif for bone targeting for intravenous therapy of hypophosphatasia, a skeletal disorder due to deficiency of alkaline-phosphatase activity (13). Similarly, fusion with the low-density lipoprotein receptor (LDLR)-binding domain of apolipoprotein resulted in an efficacy (14) and could possibly be used as a general method for delivery of lysosomal enzymes to the central nervous system.

Substrate reduction therapy is another approach that has been largely focused on lysosomal storage disorders. This approach aims at partially reducing the synthesis of the substrate of the mutant enzyme or its precursors. Miglustat, one of the first drugs developed for this goal, is not specific to a particular disease and has been used in patients with both Gaucher’s disease and Niemann–Pick type C. Eliglustat, another substrate reduction molecule evaluated for Gaucher’s disease, has shown an efficacy comparable to ERT in clinical outcomes and reduced side effects compared with Miglustat (15). Genistein instead has been shown to reduce accumulation of glycosaminoglycans in mucopolysaccharidoses (MPS) (16).

Several inborn errors of metabolism are treated by replacement of the entire organ or tissue where the mutant gene is expressed resulting in the disease. Either orthotopic or auxiliary liver transplantation has become a therapeutic option for several inborn errors of metabolism with the goal of treating the disease by replacing the defective enzyme through replacement of the whole organ. The long-term survival rates for transplanted children with inherited metabolic diseases are excellent especially when the liver is structurally normal with >90% survival after 5 years (17,18). However, liver transplantation has not completely eliminated all symptoms in patients with inborn errors of metabolism and the long-term sequelae related to immunosuppression continue to be an issue (18).

Hematopoietic stem cell transplantation (HSCT) from healthy donors has been mainly used in patients with lysosomal storage disorders based on the concept that stem cells can populate multiple tissues (including brain) and cross-correct the neighboring cells. HSCT has also been shown to be effective for peroxisomal X-linked adrenoleukodystrophy (19) although the mechanism by which transplantation arrests the disease process remains elusive. Based on currently available clinical experience, allogeneic HSCT is considered early in life for a number of lysosomal storage disorders, such as MPSI, MPSVI, MPSVII, Krabbe disease, metachromatic leukodystrophy (MLD), fucosidosis and mannosidosis (20). The number of disorders that can benefit from HSCT might expand in the future. Encouraging preclinical results have been observed in cystinosis mice in which HSCT reduced cystine in multiple organs, prevented the renal disease and improved corneal cystine crystals (21). In contrast to most lysosomal storage disorders, cystinosis is due to a defect in a transporter which cannot be corrected and up taken from neighboring cells but can be transferred from one cell to neighboring cells by intercellular vesicular transport (22). For lysosomal storage disorders with significant brain involvement, HSCT is known to be effective only in the neonatal or pre-symptomatic stages of the disease. Moreover, donor availability, high rates of graft failure, mixed chimerism and treatment-related morbidity and mortality remain important limitations of HSCT.

Novel Therapeutic Approaches

Novel applications of available drugs

As development of new drugs becomes increasingly expensive, repositioning of existing drugs has been suggested as a fast track for developing new therapies. It is likely that in the future high content screenings will provide novel applications of existing drugs. However, so far drug repurposing for inborn errors of metabolism has been mostly hypothesis-driven and here, we discuss few relevant examples.

By inhibiting an enzyme reaction that is upstream of the deficient enzyme to prevent accumulation of the toxic metabolites, nitisinone (NTBC) has revolutionized the clinical outcomes of tyrosinemia type I due to fumarylacetoacetate hydrolase (FAH) deficiency (23). With such specific inhibitory effect, NTBC had an obvious application in alkaptonuria due to defect of the same tyrosine degradation pathway. NTBC resulted indeed in decreased accumulation of the toxic precursor of the ochronotic pigment in alkaptonuria patients although clinical evidence of efficacy on joint motion have not been proven yet (24).

Sodium benzoate, sodium phenylacetate and sodium phenylbutyrate (NaPBA) are used as alternative-pathway therapies for hyperammonemia because they increase urinary excretion of ammonia through the binding with glycine and glutamine (25). Clinical experience has shown that patients with urea cycle disorders (UCD) treated with NaPBA have decreased plasma branched chain amino acids (BCAA) levels (26). In vitro and mouse studies have shown that NaPBA increases the activity of
branched-chain α-ketoacid dehydrogenase complex (BCKDC) by preventing phosphorylation of the E1α subunit mediated by BCKDC kinase (27). Thus, the resulting increased residual enzymatic activity of BCKDC leads to decreased plasma levels of BCAA in UCD patients (27). The activity of BCKDC is deficient in maple syrup urine disease (MSUD) and thus, NaPBA has been investigated in MSUD patients with the goal of reducing neurotoxic BCAA in an open-label pilot study (27). A randomized placebo-controlled study to confirm the efficacy shown by the pilot clinical study of NaPBA on plasma BCAAs in patients with MSUD is currently underway (ClinicalTrials.gov Identifier: NCT01529060). By prevention of phosphorylation-dependent inactivation of pyruvate dehydrogenase complex (PDHC), it has been shown that NaPBA also increases the enzyme activity of the PDHC and has potential for therapy of PDHC deficiency and other primary and secondary forms of lactic acidosis (28,29).

Another interesting repositioning example was driven by the observation that mTOR inhibition increases life span in mitochondrial-defective yeasts. This led to the finding that mTOR inhibitor rapamycin increased survival and weight gain, delayed mitochondrial-defective yeasts. This led to the observation that mTOR inhibition increases life span in mitochondrial-defective yeasts. This led to the identification of a number of senescence-promoting targets, such as mTOR, that can be therapeutically exploited (30). The mode of action of rapamycin is not clear. However, mTOR complex 1 (mTORC1), one of the two multi-subunit complexes that contain mTOR, was found to be activated in the brains of Ndufs4-deficient mice, and its inhibition by rapamycin conferred neuroprotection. This suggests that mTORC1 activation may contribute to disease pathogenesis in Leigh syndrome (30).

A fourth example of drug repositioning is based on the discovery of nitric oxide (NO) deficiency in argininosuccinic aciduria (ASA), a UCD due to argininosuccinate lyase (ASL) deficiency (31–33). Subjects with ASA develop long-term complications such as hypertension and neurocognitive deficits despite early initiation of therapy and the absence of hyperammonemia. Systemic hypertension in ASL-deficient mice was corrected by treatment with an exogenous NO source and NO supplements improved their neurocognitive profile. Interestingly, long-term control of hypertension and decreased cardiac hypertrophy were observed in an ASA patient treated with NO supplements (31). These pilot preclinical and clinical data show that ASA subjects could potentially benefit from NO supplementation. Hence, NO supplementation should be investigated for the long-term treatment of this condition.

Molecular chaperones and proteostasis regulators

Protein misfolding induced by missense mutations is involved in several inborn errors of metabolism and results in early degradation by the cellular protein quality control system or mis-trafficking, ultimately leading to protein loss-of-function (34). Pharmacological chaperones are small molecules that specifically bind to misfolded proteins, stabilizing their conformation, thereby preventing early degradation and allowing proper cellular trafficking and localization. These molecules that increase the activity of mutated proteins show multiple advantages, such as good safety profile and broad biodistribution to most organs and tissues including brain after oral administrations. However, not all disease-causing missense mutations are responsive to pharmacological chaperones, and increased stability of the protein detected in vitro might not be sufficient to achieve a clinical benefit. Moreover, several chaperones are inhibitors of the protein to be stabilized, generating concerns on their effect on protein activity, especially after long-term administration.

Pharmacological chaperone therapy has been largely studied for Fabry disease (35) and other lysosomal storage disorders and the reader is referred to available reviews for these applications (34). The research on cystic fibrosis (CF) had a great impact on the development of this group of drugs because the most common ΔF508 mutation in the CFTR gene impairs protein folding, function, stability and expression on the plasma membrane (36). The compound VX-809, identified by a high-throughput screening approach, partially restores CFTR function in homozygous ΔF508 patients (37). Using similar approaches pharmacological chaperones have been identified for methylmalonic acidemia (MMA) cblB type (38) and primary hyperoxaluria type 1 (PH1) (39). PH1 is due to deficiency of the hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT) leading to accumulation of toxic oxalate that results in end-stage chronic kidney disease and systemic damage secondary to tissue deposition of calcium oxalate. Missense mutations in the gene encoding AGT affect the conformation of the enzyme, decrease protein stability and lead to mistargeting of the peroxisomal protein toward mitochondria (40). A screen of small-molecule modulators that attenuate AGT mitochondrial protein translocation, led to the identification of a Food and Drug Administration-approved small molecule, dequalinium chloride, which restores trafficking of AGT from mitochondria to peroxisomes with subsequent reduction in oxalate production (39).

Another class of molecules able to influence the fate of misfolded proteins is the proteostasis regulators, which facilitate protein folding and minimize misfolding by increasing the function and availability of molecular chaperones and/or by activation of the protein quality control system (41). Examples of proteostasis regulators include: geldanamycin that up-regulates molecular chaperones, anti-epileptic drug carbamazepine that inactivates histone deacetylases inhibitors that exert pleiotropic effects and the proteasome inhibitor bortezomib that can either reduce the levels of aberrant toxic proteins or prevent degradation of still functional variant proteins (42).

Read-through drugs

Read-through drugs allow the ribosome to selectively read-through non-sense mutations to generate functional proteins by preventing mRNA degradation by non-sense-mediated decay (43,44). Ataluren (or PTC-124) is a drug with translational read-through capacity that has been extensively investigated in preclinical models and in clinical trials (45) mostly for Duchenne muscular dystrophy (DMD) and CF. In a phase II randomized, double-blind, placebo-controlled trial in DMD patients, the drug was well tolerated but the efficacy data lack of robustness (45). The use of Ataluren has been proposed for the treatment of patients with a variety of inborn errors of metabolism (46–50), and a clinical trial has recently been performed for MMA (ClinicalTrials.gov Identifier: NCT01141075) (51). However, the results of this trial still have to be disclosed. An important limitation for read-through drugs is the potential immune reaction against the unrecognized protein product induced by the drug (43).

Cell therapies

To overcome some of the limitations of liver transplantation, hepatocyte transplantation has been investigated for several inherited metabolic diseases and Crigler–Najjar syndrome was the first disorder in which this procedure was attempted (52). The cells obtained by enzymatic dissociation with collagenase of the donor
liver were separated from the non-parenchymal fraction by centrifugation and delivered via a portal vein catheter that is far less invasive than orthotopic liver transplantation. In contrast to orthotopic liver transplantation, this therapy could be performed safely in severely ill patients, such as patients with acute metabolic decompensation. In the presence of a structurally normal host liver, a fraction of transplanted cells should cross the endothelium to integrate into the host liver. However, the engraftment of transplanted hepatocytes is limited by the normal liver architecture and normal cell viability in most inherited metabolic liver diseases. Therefore, liver repopulation by normal-functioning hepatocytes only occurs if recipient hepatocytes are affected by a cytotoxic disease process or are removed, and if cells have a selective growth advantage over uncorrected cells. For these reasons, extrahepatic transplantation sites such as lymph nodes are currently being pursued for liver cell therapy (53).

The percentage of liver cell replacement needed to achieve a clinical benefit varies with the disease and some disorders require higher percentage compared with others depending on the pathophysiology and on the magnitude of metabolic flux through the impaired enzyme reaction. In Crigler–Najjar patients, the infusion of 5% of the estimated liver mass was sufficient to decrease significantly the bilirubin level (52,54,55). In most diseases, the percentage of liver mass to be injected to achieve clinical benefit is about 10% of liver mass. Results of hepatocyte transplantation in familial hypercholesterolemia (FH) and organic acidemias have been less encouraging suggesting that these disorders might require higher percentage of cells. Hepatocyte transplantation has been performed so far in several patients with metabolic diseases, and the results of published cases, which are usually single patient reports, have been recently reviewed (56). Overall, the major limitation of hepatocyte transplantation emerging from these cases is the duration of correction that is transient and is generally lost within 12–18 months from the infusion (57). A clinical trial to investigate more systematically the safety and efficacy of hepatocyte transplantation is currently ongoing for UCD and Crigler–Najjar syndrome (ClinicalTrials.gov Identifiers: NCT00718627 and NCT01345578) (57).

An alternative approach to generate hepatocytes for therapeutic applications is to use stem and/or progenitor cells, which have a high capacity for expansion and can be amplified and differentiated into the desired cell types. Pluripotent stem cells, including human embryonic stem cell and induced pluripotent stem cell (iPSC) lines, have a high proliferative capacity and can differentiate in vitro and in vivo into diverse lineages, including hepatocyte-like cells that exhibit several phenotypic and functional features of mature hepatocytes (58–60). Furthermore hepatocyte-like cells can be derived by direct differentiation of fibroblasts and, like iPSCs, might provide an autologous stem cell source that would bypass the need of immune suppression (61–64). Transplanted hepatocyte-like cells obtained through this transdifferentiation were able to repopulate the livers of FAH-deficient mice (61). Notably, transdifferentiated hepatocyte cell transplantation would not carry the risk of teratoma formation from contaminating iPSCs, which has hampered the clinical application of pluripotent stem cell-derived hepatocytes.

Another application of iPSCs was the generation of liver buds from partially differentiated human iPSCs mixed with supportive stromal and endothelial cells to mimic early liver development. By this approach, instead of introducing iPSC-derived hepatocytes into a hostile microenvironment, the cells were delivered into extrahepatic sites as self-contained organoids mimicking embryonic liver. The liver organoids were able to vascularize upon ectopic transplantation and rescued a mouse model of drug-induced liver injury (65). This type of approach has potential for therapy of non-cell autonomous inherited diseases that can be corrected by ectopic expression of the defective metabolic pathway.

**Ex vivo gene therapy**

Inborn errors of metabolism are monogenic disorders and in principle the replacement of the defective gene would provide a definitive cure for these disorders. Therefore, several inherited metabolic diseases have been the objective of gene therapy investigations in both ex vivo and in vivo approaches. In ex vivo approaches, the patient’s cells are removed from the patient and after gene correction they are injected into the patient.

Ex vivo gene transfer of hematopoietic stem cells can now be considered as at least as effective to allogenic HSCT based on results of clinical gene therapy trials in patients with XL-ALD and MLD (66,67). However, in contrast to allogenic HSCT, the autologous cells eliminate the need for searching for an appropriate donor and the risks of graft-versus-host disease. It is hypothesized that over time microglial cells derived from gene-modified hematopoietic stem cells replace resident microglial cells and provide both local and systemic replacement of the deficient enzyme. For the MLD gene therapy trial, an optimized lentiviral vector-mediated gene transfer resulting in supraphysiologic enzyme levels led to an impressive halt in the disease progression with improvements in brain imaging, electrophysiological measurements, biochemical parameters and disease progression compared with previous affected siblings in each family. Genomic integration analysis showed robust poly-clonality of graft cells in different blood lineages. However, the evaluation of safety is still early and besides genotoxicity, the additional concern about potential long-term toxic consequences of higher than normal levels of enzyme has to be addressed. In addition, so far ex vivo gene therapy will be limited to those cases that are identified pre-symptomatically based on family history, until MLD is included into newborn screening programs.

Ex vivo gene replacement of hepatocytes are desirable as an alternative to hepatic transplantation in inborn errors of metabolism because it would eliminate the need of life-long immune suppression. This approach has been investigated in a patient with FH: autologous hepatocytes removed through a partial hepatectomy were transduced ex vivo with a retroviral vector bearing the LDLR gene and then re-implanted in the patient (68). Nevertheless, this attempt only resulted in transient reduction of hypercholesterolemia and had a number of shortcomings including limited engraftment of genetically modified hepatocytes, limited viability of cultured primary hepatocytes and the need of invasive procedures to obtain hepatocytes, and their reinfusion in the portal vein following ex vivo gene replacement.

**In vivo gene replacement therapy**

Given the limitations of ex vivo gene therapy, gene therapy for inherited metabolic diseases is mostly based on in vivo approaches. In the in vivo approaches, the gene therapy vector is directly injected into the organism by either systemic (intravenous, portal vein injections) or localized (intramuscular, intracerebral) injections. A large number of virus-derived gene transfer vectors have been developed and investigated in preclinical models but so far the adeno-associated viral (AAV) vector has emerged as the most promising based on its efficacy and safety that have been recently confirmed also in human trials. AAV vectors...
transduce non-dividing cells and result in long-term expression of the transgene with low toxicity but are limited by a small cloning capacity.

The encouraging results from a gene therapy trial using AAV serotype 8 (AAV8) in hemophilia B patients indeed has sparked new enthusiasm for development of liver-directed gene therapy for inborn errors of metabolism (69,70). In this trial, a single intravenous infusion of vector in patients with severe hemophilia B resulted in a dose-dependent increase in circulating factor IX (FIX) to therapeutic levels that were sustained long-term, allowing reduction of the need for prophylactic FIX infusions and decreasing bleeding episodes in the group of patients receiving the higher vector dose. However, a mild increase in alanine aminotransferase due to a cytoxic T lymphocyte (CTL) immune response occurred between 7 and 10 weeks post-vector administration in four of the six patients in the high-dose group that resolved after prednisolone treatment (69,70). Similar to hemophilia B, Crigler–Najjar syndrome likely requires a small percentage of corrected hepatocytes (~5%) to achieve clinical benefit, as suggested by hepatocyte transplantation studies (52). Patients with Crigler–Najjar syndrome type 1 are refractory to phenobarbital treatment, experience life-threatening elevations of bilirubin and are generally managed with phototherapy throughout childhood and adolescence. Although effective, phototherapy is cumbersome, inconvenient, significantly impairs the quality of life and diminishes its efficacy with time as a consequence of increased skin thickness and decreased surface/mass ratio. Moreover, despite treatment, patients remain at risk for brain damage when intercurrent infections increase levels as bilirubin above those which are controllable by phototherapy (71). Therefore, patients with Crigler–Najjar syndrome type 1 are often advised to consider liver transplantation. Although the onset of the hyperbilirubinemia occurs early in life, AAV administration can be postponed to adolescence or early adulthood to overcome the issues related to liver growth and insertional carcinogenesis which are further discussed below. For these reasons, Crigler–Najjar disease appears a good candidate for AAV8-mediated liver-directed gene therapy.

Several inborn errors of metabolism have an acute onset in the newborn period and successful treatment of severe neonatal disease is considerably difficult: rapid onset of acute decompensations makes it difficult to achieve adequate levels of transgene expression with sufficient rapidity, despite vector delivery soon after birth. Moreover, loss of vector genomes due to liver growth requires vector re-administration which is hampered by the immune responses induced against the vector. Several studies have consistently shown loss of episomal AAV vector genomes as a consequence of liver growth (72,73). The decline in transgene expression, particularly during the early rapid growth phase of dividing tissues, is a significant obstacle that compromises long-term phenotypic correction. The human liver doubles in weight between the newborn period and the first 3 months of life, doubles again by 10 months and then once more by the age of 5 years (74). Loss of episomal vectors is a major challenge for inborn errors of metabolism requiring treatment early in life. Moreover, early timing of gene delivery appears to be a critical factor for genotoxicity and risks of hepatocellular carcinoma (HCC) after AAV gene delivery at least in mice (75–77).

The risks of insertional carcinogenesis following systemic AAV administration are indeed a concern. The first evidence of such issue came from a study in MPSVII mice that were found to develop HCC following neonatal AAV injections (76). The authors observed integration of the vector genome locus on chromosome 12. Some authors have postulated that the effect was related to the expression of the LacZ transgene more than to the vector administration (78). However, these findings were also observed more recently in MMA mice, which also developed HCC several months after neonatal AAV injections and showed vector integration and overexpression of microRNA-341 (Mir341) proximal to the RNA imprinted and accumulated in nucleus (Rian) locus, in the same locus on chromosome 12 (75). Similarly, a higher frequency of HCC was observed in molybdenum cofactor deficient mice injected with AAV as newborns (77). In contrast, no evidence of insertional mutagenesis and cancer were observed in adult mice for 18 months (79), in dogs for a period of 8 years (80) and in non-human primates for up to 5 years (81). It should also be noted that in mice which developed cancer the AAV integrated in the Mir341 within the Rian locus that has no orthologs in the human genome. The possibility of insertional carcinogenesis in humans remains an open question, and this hypothetic risk represents a factor to be considered in the risk–benefit ratio evaluation.

The muscle has been a preferred tissue for gene therapy because of its simple access through intramuscular injections and its safety. Muscle-directed gene therapy with AAV vectors has generated encouraging preclinical results in hemophilia B mice and dogs. These studies have led to a human clinical trial, which has shown excellent safety data and evidence of gene transfer. However, no clinical benefit was observed (82). In a trial investigating intramuscular administrations of AAV vectors in patients with lipoprotein lipase deficiency (83), loss of correction in trygliceride plasma levels was associated with a transient increase in serum creatine phosphokinase in the high vector dose cohort, as a consequence of a CTL response against AAV capsid proteins, similarly to what observed in the hemophilia B clinical trial (84,85). Patients receiving higher doses were then also treated with immunosuppressive drugs from the time of vector administration and up to 12 weeks post-injection. Overall, vector administration was well tolerated and was associated with sustained transgene expression, reduction of fasting plasma tryglicerides, long-term changes in trygliceride-rich lipoprotein characteristics and fewer pancreatitis attacks (86–88). The results of this trial led to official approval by the European Medicine Agency of Glybera (alipogene tiparvovec) as the first gene-therapy medicine to be recommended for authorization in the European Union (89).

RNA targeting

Targeting the genetic defects at the RNA level has potential for therapy of several disorders including inborn errors of metabolism. This approach is based on molecules that bind nucleic acids with high specificity and modulate mRNA metabolism (90). Typically, RNA interference (RNAi) are double-stranded RNAs (dsRNAs) of which one strand has a sequence complementary to that of a messenger RNA (mRNA) resulting in reduction or elimination of a target mRNA. The dsRNAs can be provided as synthetic oligonucleotides or as genetic DNA templates from which the RNAi triggers are transcribed in the target cells (vector-based transcriptional RNAi). RNAi has been successfully applied in transnhyretin amyloidosis and various RNAi compounds are in clinical trials (91,92). Mipomersen is an antisense oligonucleotide that inhibits the liver production of human apolipoprotein B and reduces the serum levels of low-density lipoproteins in patients with homozygous FH (93,94).

Gene editing

While gene replacement strategies are entering the phases of clinical development, novel technologies, such as zinc-finger
nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, have recently been developed and have the potential for becoming the next generation of gene therapy for inborn errors of metabolism. Targeted genome editing mediated by ZFN performed ex vivo to induce knockout of the CCR5 gene encoding for human immunodeficiency virus co-receptor has shown safety in human clinical trial (95), and similar approaches have been applied for correction of genetic defects in human stem cells (96,97). Moreover, a proof of concept for correction of type 1 tyrosinemia in mice using CRISPR/Cas9 technology has also been provided. In this study, delivery of the components of the CRISPR/Cas9 system to the liver by tail vein hydrodynamic injection induced correction of a single base mutation of the Fah gene in up to 36% hepatocytes. The correction was accompanied by reduction of liver damage and improved survival (98).

Although these approaches are still far from being used in clinical applications because of significant issues related to safety and delivery, they have the potential to overcome the issue of loss of transgene expression due to hepatocyte division and might permit gene expression within its physiologic genomic context.

**Concluding Remarks**

Treatment of inborn errors of metabolism is a challenge. These disorders are severe and available treatments are often disappointing. Nevertheless, the repertoire of drugs is steadily increasing with a number of therapeutic strategies that we could not even imagine a few years ago. By tackling the enzyme deficiency through multiple and different approaches, there is hope for developing more effective therapies in the near future for several inborn errors of metabolism.

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