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Development of precision therapies for rare inborn errors of metabolism: Functional investigations in cell culture models

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Abstract

Due to the low number of patients, rare genetic diseases are a special challenge for the development of therapies, especially for diseases that result from numerous, patient-specific pathogenic variants. Precision medicine makes use of various kinds of molecular information about a specific variant, so that the possibilities for an effective therapy based on the molecular features of the variants can be elucidated. The attention to personalized precision therapies has increased among scientists and clinicians, since the "single drug for all patients" approach does not allow the classification of individuals in subgroups according to the differences in the disease genotype or phenotype. This review article summarizes some approaches of personalized precision medicine that can be used for a cost-effective and fast development of therapies, even for single patients. We have focused on specific examples on inborn errors of metabolism, with special attention on drug repurposing. Furthermore, we provide an overview of cell culture models that are suitable for precision medicine approaches.

KEYWORDS

drug repurposing, inborn errors of metabolism, pathogenic variants, personalized medicine, precision medicine, rare diseases

1 | PERSONALIZED PRECISION THERAPIES FOR RARE INBORN ERRORS OF METABOLISM

There are more than 8000 rare diseases, over 80% of which exhibit a genetic component. Due to their rareness, these diseases are often neglected in terms of research on treatments, as the global patient population is considered too small for drug development to be financially interesting for pharmaceutical industry. Therefore, treatment options that would be suitable for most or even all patients with a specific disease, such as gene therapy or enzyme replacement therapy (ERT), are either not addressed at all, or they never proceed from preclinical studies to clinical trials due to lack of interest of pharmaceutical companies. Furthermore, gene therapy and ERT, even if they become available for a specific disease, are very expensive therapies that may not be accessible to patients in underdeveloped countries.

Miroslava Didiasova and Antje Banning contributed equally to this study.

Precision medicine is an emerging approach that makes use of various kinds of molecular (e.g., genetic, transcriptomic, proteomic, metabolomic, etc.) information about a patient to assess the possibilities for an effective therapy. The attention to precision medicine has been growing in recent times, especially among scientists and clinicians, who have realized that the conventional therapy or "single drug for all patients" approach does not allow the classification of individuals in subgroups according to the natural variations present in the disease genotype or phenotype.

Whereas some monogenic diseases exhibit predominant variants of the disease-specific gene that are found in a majority of patients, most genetic diseases result from a number of heterogeneous genetic changes. Thus, the patients typically have various, many times family specific, mutations, either in a compound heterozygous or homozygous manner. Nevertheless, different disease-causing mutations may have a considerable impact on disease severity and on the response to a certain treatment. An essential first step in drug development is a thorough characterization of the molecular consequences of each disease-causing variant at mRNA and/or protein level. Important questions are, for example, how does the variant influence the folding, processing and cellular transport of the affected protein? Are posttranslational modifications affected? Is the patient variant still stable, and how severe is the folding defect caused by a missense variant? In case of an enzyme, it is important to study how the enzyme activity is impaired, and if there is some residual enzyme activity left. For variants directly affecting the catalytic residues, some treatment approaches may not work, even if the protein is expressed in normal amounts, because the enzyme catalysis as such is impaired. In some cases, an altered proteolytic processing may result in a functional impairment of an enzyme.[1,2]

In this review article, we will focus on therapy approaches different from the "single drug for all patients" strategy. Below, we will outline a drug development strategy for rare diseases that is relatively inexpensive, as it is frequently based on drug repurposing with small molecules. These molecules can function by supporting the folding and transport of the mutated protein (for missense variants), or they can influence the mRNA splicing (for splice site mutations), or produce a translational read-through (for nonsense variants). For this kind of drug development, it is essential to characterize and understand the molecular consequences of each pathogenic variant, some of which may only be found in a small number of patients, or even in only a single patient. Therefore, this kind of a drug development approach is a prime example of highly personalized precision medicine. The purpose of this article is not to review all existing approaches and diseases or to give a complete review of the literature. We will use specific examples of drugs and diseases, hoping to encourage similar research on further neglected or orphan diseases, thereby boosting the development of precision treatments for rare diseases.

2 | CELL CULTURE MODELS

For identifying the best treatment option for a given patient with a specific disease-causing variant, cellular models can help developing a personalized therapy for the patient or even for a group of patients that share the same variant. These models and their use in drug development are depicted in Figure 1. Three different model systems for preclinical studies are mainly used, all of which have their pros and cons: (1) patient-derived fibroblasts, lymphoblasts or other primary cells that may be immortalized; (2) engineered cell lines with constitutive (over)expression of a specific gene variant; (3) patient-derived, induced pluripotent stem cells (iPSC) or other reprogrammed patient-derived cells. Some of the cell models can be used in three-dimensional cell culture systems, or organoids can be generated. These models better reproduce the organ-specific disease phenotypes than standard two-dimensional cell models.[3]

To maximize the likelihood of therapeutic success, patient cells, or derivatives thereof, should generally be the first choice for carrying out pharmacological screenings. Once all required permissions are available, patient fibroblasts can be obtained from a skin-puncture biopsy.[4] Fibroblast cultures are easily established within 2 weeks by growing the dissected skin pieces in a suitable culture medium. Thereafter, a sufficient number of stocks should be frozen down, due to the limited period of growth of such primary cells, which is the major drawback of working with patient-derived primary cells. Fibroblasts can be cultured for ~1 month in standard, low-cost cell culture medium. However, not all fibroblast cultures grow equally well, especially if the donor is beyond her/his teens. For data analysis, fibroblasts from healthy donors should be used for comparison. Overall, patient fibroblasts are the most common model for successful precision medicine and can provide fast answers to questions such as response of a particular variant to a specific drug. Similarly, lymphoblast cell lines can be created by infecting peripheral blood lymphocytes with Epstein–Barr virus or other viruses.[5] Repositories, such as the Coriell Institute for Medical Research and WiCell, provide a large collection of various patient-derived cells, including fibroblasts, lymphoblastoid cell lines, iPSC, and age- and sex-matched controls from healthy donors.[6,7]

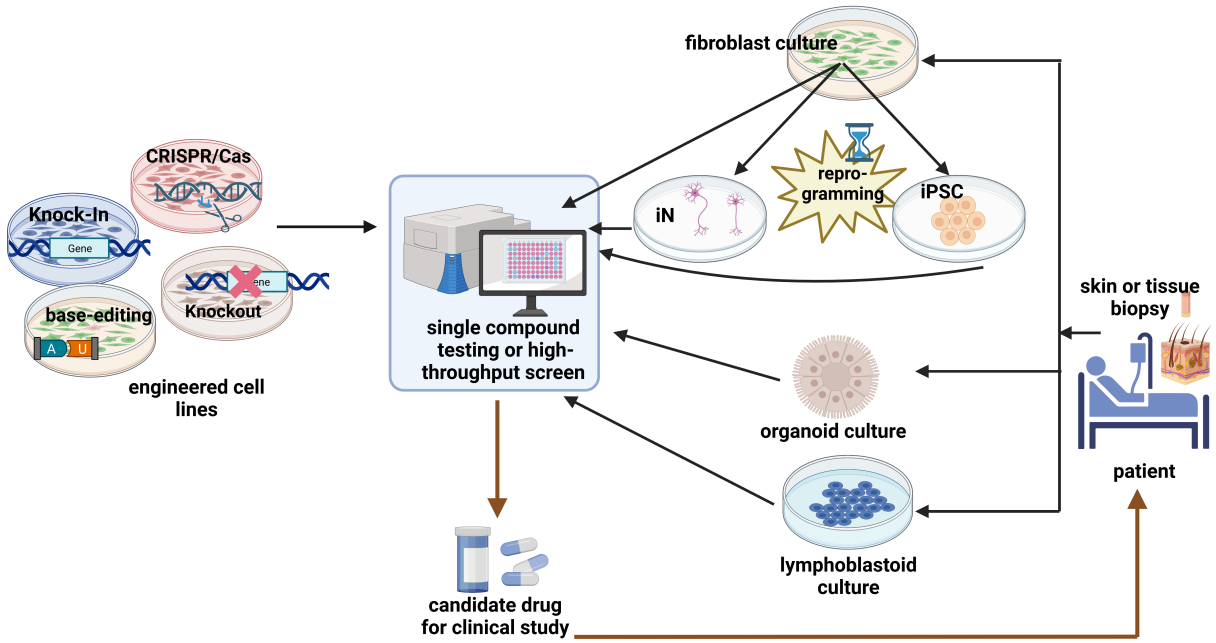

FIGURE 1 Potential cell models and their use for the development of precision therapies for rare neurometabolic diseases. Patient-derived cells such as dermal fibroblasts can be reprogrammed to induced pluripotent cells (iPSC) or induced neuronal cells (iN). Organoids can be established from tissue biopsies, and lymphoblastoid cultures are derived from immortalized white blood cells. Human cell lines can be engineered so that they contain a knockout of the disease genes and/or a knock-in of patient-specific variants. Compound testing or screening performed with these models will reveal candidate drugs that can be further verified and tested in clinical studies (image created with BioRender.com).

One of the most impressive examples for developing a novel therapeutic agent for a single pathogenic variant found in only one patient was the invention of Milasen, a customized splice-modulating antisense-oligonucleotide that was designed for a patient affected with the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis type 7.[8] This novel drug was developed within 10 months, including all necessary authorizations, using patient-derived skin fibroblasts as a model system,[8] which is exceptionally fast. More typical is the repurposing of approved drugs that are often identified by compound screens, for example, by using platforms such as Braincure/Mitocure/Myocure[9] or by targeted testing of candidate drugs.[10–12]

For high-throughput screening of multiple compounds, appropriate readouts and high number of cells are needed, which is typically limited when using primary fibroblasts. Hence, robust cell lines in which the gene of interest is modified to mimic a patient specific, disease-causing mutation are advantageous. Over the last decade, several genome editing technologies were developed to first produce a knockout of the endogenous gene, and then to replace it with the mutated version, or to modify it by a base editing approach.[13] For instance, Flp-In recombinase systems[14] or integration of genes into the AAVS1 safe harbor locus[15] are versatile tools for generating cell lines with targeted, constitutive overexpression of the gene variant of interest.

Both fibroblasts and constitutive cell lines are of limited use when certain cell types, for example, neurons or muscle cells, need to be analyzed. For such purposes, patient-derived organoids[16] or reprogramming of patient-derived fibroblasts into iPSC or directly into other cell types can be the model of choice (for a review, see Ref. 17). The clear advantage of iPSC is their pluripotency that allows the generation of cell types that cannot be obtained directly from the patient. However, the high complexity of the method, genetic instability, risk of generating tumorigenic cells, aberrations of mitochondrial DNA and high costs are obstacles on the way of these models (for a review, see Ref. 18). Nevertheless, iPSC were successfully developed and are valuable tools for personalized medicine approaches, as was demonstrated for single patient variants of sialidosis,[19] CLN5,[20] Gaucher disease (reviewed in Ref. 21), and many others.[22–24] Moreover, iPSC can be combined with simultaneous base editing, showing potential for future therapies based on autologous cell transplantation of in vitro-corrected patient cells.[13] A very elegant approach is the direct reprogramming of fibroblasts to neurons (iNs)[25,26] or, as a future technology, to any other cell type (e.g., hepatocytes).[27] This technology has made

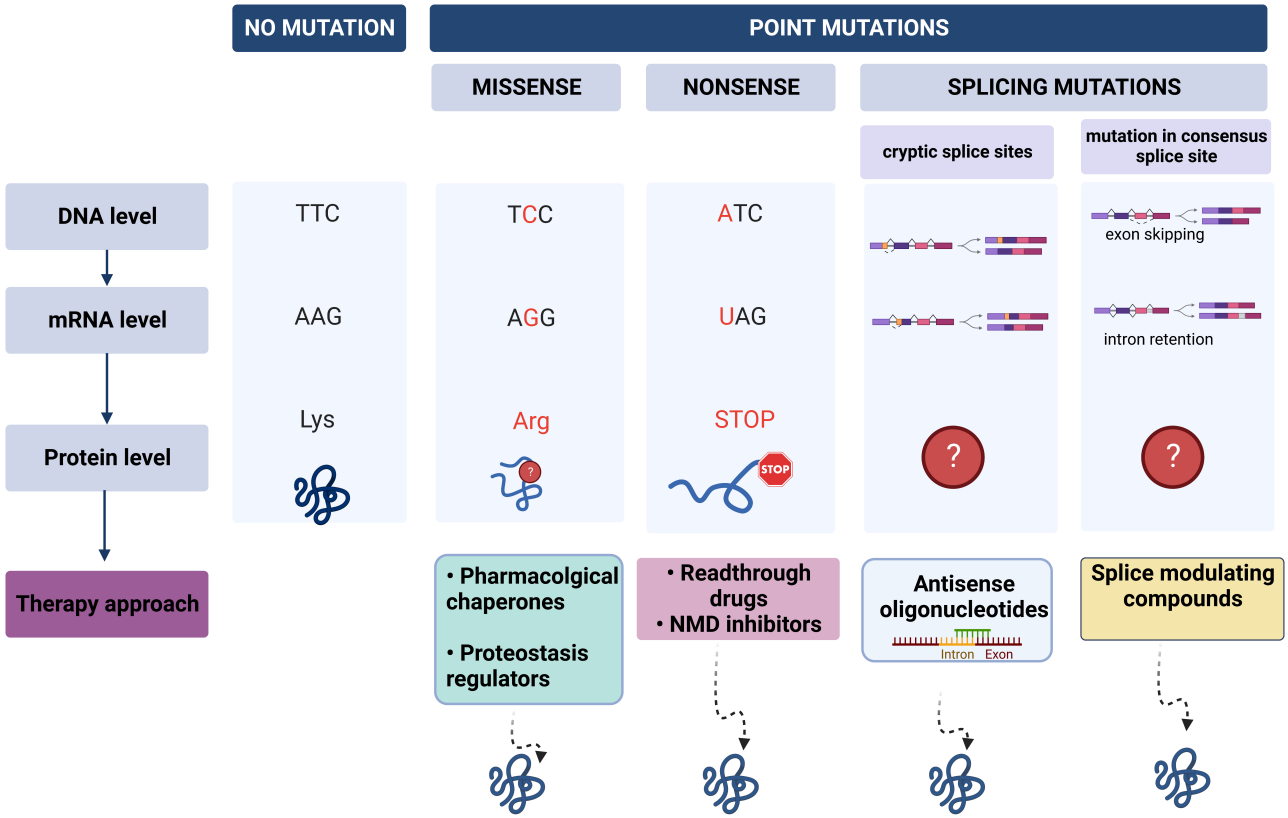

FIGURE 2 Options for therapy approaches based on the type of the genetic variant. Depending on the type of the patient variant (nonsense, missense, splicing variants), various approaches are available (see text for details). These approaches can be tested after a thorough characterization of the consequences of the variant at DNA, RNA, and protein level. NMD, nonsense-mediated decay (Adapted from the template "Point Mutations" of BioRender.com. Retrieved from https://app.biorender.com/biorender-templates.).

substantial progress over the last years, but it still struggles with efficiency and purity issues (reviewed in Ref. 9). Therefore, these approaches have so far mainly focused on cells from healthy donors. Direct reprogramming of patient cells into iNs was shown for Krabbe disease,[28] supporting the proof of concept of this technology.

3 | PRECISION THERAPIES BASED ON VARIANT TYPE

Because precision medicine uses a different approach than the "single drug for all patients" strategy, precise knowledge about the molecular features of the disease-causing variants is mandatory. Variant-specific approaches are shown in Figure 2. Different pathogenic variants may show a varying response to a specific drug, and interindividual variations in further genes may contribute to the drug response. Disease-causing missense variants that lead to misfolding, misprocessing, or instability of the protein exhibit the greatest potential for developing a variant-specific treatment that is based on improving protein folding or stability. Here, the chance of identifying a compound that either prolongs the half-life of an unstable protein or improves the folding of the protein is much higher, as compared to variants that are caused by, for example, frameshift mutations, insertions, or deletions.

Proteins that show local folding defects may benefit from chaperone therapies that target protein folding. In contrast to unspecific chemical chaperones, such as glycerol or dimethyl sulfoxide that are not useful for the treatment of patients, the concept of pharmacological chaperone (PC) therapy aims at specifically stabilizing misfolded proteins by helping them during folding and by promoting the trafficking to their final destination (for a review, see Ref. 29–31). A prerequisite for a PC compound is that it (reversibly) binds to its target protein, stabilizing its correct structure. In line with this, PCs often are inhibitory molecules or agonists of the target protein.

PC therapy has shown significant promise in many diseases, and several drugs have been repurposed as PCs, including the mucolytic substances ambroxol in Gaucher disease[32,33] and acetylcysteine in Pompe disease,[34] the antiparasitic pyrimethamine in GM2 gangliosidosis,[35,36] and the flavone diosmetin in Zellweger spectrum

disorder.[37] The iminosugar Migalastat was originally discovered as a potential drug for Fabry disease in patient-derived lymphoblasts,[38,39] and it is now in clinical use for Fabry patients.[40] Our own research identified betaine (tri-methyl glycine) as a PC molecule suitable for the world-wide most common pathogenic variant in aspartylglucosaminuria (AGU).[10] This drug, also known as Cystadane®, is approved for the treatment of homocystinuria, and our cell culture studies thus swiftly advanced into a clinical trial.[41] The discovery of these drugs confirms the validity of patient-derived primary cells as important tools for preclinical research.

In addition to PCs, the use of proteostasis regulators is a promising approach (reviewed in Ref. 29). These substances can either enhance the expression or function of endogenous molecular chaperones, mainly heat-shock proteins (HSP) such as HSP70, HSP90, and HSP40, or inhibit either proteasomal degradation or endoplasmic-reticulum-associated protein degradation. Proteasomal inhibitors such as bortezomib, an FDA-approved drug for multiple myeloma, turned out to reduce cholesterol accumulation in fibroblasts of Niemann-Pick type C patients.[42] Similarly, the heat shock response activator celastrol showed promising results in Gaucher disease and Tay Sachs disease fibroblasts,[43] while arimoclomol, an orally available coinducer of especially HSP70,[44] was already tested in clinical trials in Niemann-Pick type C patients[45] and in amyotrophic lateral sclerosis (ALS).[46] Unfortunately, recent data from the Phase 3 trial demonstrate that arimoclomol failed to show efficacy in ALS.[47]

While missense variants may respond to drugs that aid protein folding or stabilization, such approach is not applicable for patients with variants that contain premature stop codons (nonsense variants) or variants that affect pre-mRNA splicing. For nonsense variants, which account for up to 20% of disease-causing single nucleotide substitutions and for 11% of all gene variants that cause inherited human diseases,[48] substances that inhibit nonsense-mediated decay (NMD) of mRNA (e.g., caffeine) and/or induce a translational read-through (e.g., amlexanox, aminoglycosides) showed great promise. Such substances could potentially be used in many patients with a similar type of variant, independent of the disease or the affected gene. However, even the well-studied candidate drug, ataluren (PTC124, Translarna™)[49–52] that obtained EMA-approval for Duchenne muscular dystrophy in 2014, could not convince in clinical trials.[53] So far, all tested read-through-substances showed heterogeneous results, mainly because the amount of read-through may be limited by the unstable mRNA undergoing NMD, or because the substance does not reach the target cell or organ. For example, while amlexanox was promising in AGU patient fibroblasts,[54] successful treatment of patients would require that the compound can cross the blood-brain barrier. Recently, 2,6-diaminopurine was shown to effectively promote efficient read-through of UGA premature stop codons,[55,56] and the NMD-inhibitor caffeine was able to boost ataluren's read-through capacity.[57] These findings give hope for the continuation of research on read-through treatments, perhaps combined with NMD inhibitors, for nonsense variants.

RNA splicing is a key regulatory step in gene expression, and splicing variants account for up to 15% of disease-causing mutations.[58] Depending on the type and position of the nucleotide exchange that can happen in either exons or introns, various types of consequences are possible. These mutations can lead to activation of cryptic splice sites, skipping of exons, or retention of introns, all of which typically result in aberrant transcripts and profound reduction or absence of correctly spliced mRNA. Splicing mutations were for long regarded as untreatable. Meanwhile, cryptic splice sites or newly formed splice sites have successfully been targeted with antisense oligonucleotides (ASOs) that mask the abnormal splicing sites and produce alternatively spliced protein variants that may have sufficient residual function.[59–61] However, this approach requires the design of a special ASO for each individual variant. In some diseases, a certain variant is present in the majority of the patients, like in Pompe disease, where two thirds of the late onset cases are caused by the same splicing variant (i.e., c.-32-13 T > G). Thus, these patients are expected to benefit from the same ASO.[62]

Mutations in the consensus sequences at the borders of introns and exons also lead to faulty splicing of the pre-mRNA, because the exon/intron boundaries are not properly recognized by the spliceosome. If there were ways to improve the recognition of such mutated consensus splice sites, correct splicing of at least a fraction of the pre-mRNAs could be achieved, which could increase the synthesis of the normal protein and thus be of great benefit for many diseases. Different orally available small molecules that function as splicing modulator compounds and enhance the recognition of nonstandard splice donor or acceptor sites have been identified. The plant hormone kinetin has been shown to enhance the splicing of mutated splice donor sites,[63] while the flavonoids luteolin and apigenin improve splicing at mutated acceptor sites.[1,64,65] This effect can further be enhanced when these substances are combined with caffeine[1] that is known to inhibit NMD and to regulate splice factor expression.[66] The FDA-approved iron chelator deferoxamine that was identified by a compound library screen was shown to be potentially useful for the common

splicing variant Pompe disease.[67] The exact mechanism of deferoxamine action is not clear, but it could involve the inhibition of the splicing regulator serine/arginine-rich splicing factor 7 (SRSF7) by increased levels of iron.[67]

Recently, a compound that suppresses aberrant splicing of the cystic fibrosis *trans*-conductance regulator (CFTR) pre-mRNA at a pseudoexon splice donor site was identified in a chemical library screen.[68] The compound named CaNDY (CDC37 association inhibitor for (Z)-5-[(2,3-dihydrobenzofuran-5-yl)methylene]-2-iminothiazolidin-4-one)) inhibits CDC-like kinase, an activator of SRSFs that are required for pseudoexon recognition.[68] Hence, CaNDY might also be a candidate drug for other pseudoexon variants.

Splice-modulating compounds have the potential for working for a number of diseases, but the discovery of sequence motifs that are likely to be responsive to a certain compound remains a challenge. However, BPN-15477, a potent modulator of mRNA splicing in familial dysautonomia, was assessed using a deep-learning approach to identify other disease-causing variants that might respond to treatment with this compound.[69] Impressively, the authors were able to identify 214 annotated disease-causing variants in 155 genes that could be targets for splicing correction using BPN-15477.[69] This underscores the value of suitable data analysis tools for the increasing number of annotated gene variants and for the discovery of therapeutic targets for treating human genetic diseases.

4 | CONCLUSIONS

Precision therapy approaches based on repurposing of small molecules provide an accessible and economic treatment option for rare metabolic diseases. Some research has already been conducted to identify drugs that can be repurposed for specific diseases and certain pathogenic variants. These studies are frequently complicated by the presence of numerous patient-specific variants, all of which may not respond to the same treatment. In addition, most of the animal models available for drug testing are gene-knockout animals that are useless for testing PC, read-through, or splice-modulator substances. Since producing knock-in mouse models for a large number of patient variants is not feasible, such animal models could be replaced by, for example, patient-derived organoids obtained from iPSC cells.[17] Such models would allow the preclinical testing of substances identified in experiments using more simple models, such as patient fibroblasts, before the drug is applied to a patient. Since drug repurposing can, in principle, be done without animal studies, this approach would speed up the personalized precision drug development at low cost.

AUTHOR CONTRIBUTIONS

Miroslava Didiasova and Antje Banning contributed equally to this work. Antje Banning made the figure drafts. All authors participated in article drafting and editing. All authors read and agreed with the final article version.

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CONFLICT OF INTEREST STATEMENT

Miroslava Didiasova and Antje Banning declare that they have no conflict of interest. Ritva Tikkanen has received research funding from several patient organizations and pharmaceutical companies for research related to various inborn errors of metabolism.

DATA AVAILABILITY STATEMENT

The article does not contain any experimental data.

ETHICS STATEMENT

This article does not contain any studies with human or animal subjects performed by any of the authors.

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