



**Repositorio Institucional de la Universidad Autónoma de Madrid**

<https://repositorio.uam.es>

Esta es la **versión de autor** del artículo publicado en:

This is an **author produced version** of a paper published in:

Clinical Genetics 93.3 (2018): 450-458

**DOI:** <http://doi.org/10.1111/cge.13088>

**Copyright:** © 2017 John Wiley & Sons A/S

El acceso a la versión del editor puede requerir la suscripción del recurso

Access to the published version may require subscription

## REVIEW

Protein misfolding diseases: prospects of pharmacological treatment

Alejandra Gámez<sup>1</sup>, Patricia Yuste-Checa<sup>1</sup>, Sandra Brasil<sup>1</sup>, Álvaro Briso-Montiano<sup>1</sup>,  
Lourdes R. Desviat<sup>1</sup>, Magdalena Ugarte<sup>1</sup>, Celia Pérez-Cerdá<sup>1</sup> and Belén Pérez<sup>1\*</sup>

<sup>1</sup>Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular-SO UAM-CSIC, Universidad Autónoma de Madrid, Campus de Cantoblanco, 28049 Madrid/Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Investigación Sanitaria IdiPAZ, Madrid, Spain.

\*Correspondence: B. Perez (bperez@cbm.csic.es; Tel: +34 91 196 45 66)

## Abstract

Protein misfolding has been linked to numerous inherited diseases. Loss- and gain-of-function mutations (common features of genetic diseases) may cause the destabilization of proteins, leading to alterations in their properties and/or cellular location, resulting in their incorrect functioning. Misfolded proteins can, however, be rescued via the use of proteostasis regulators and/or pharmacological chaperones, suggesting that treatments with small molecules might be developed for a range of genetic diseases. This work describes the potential of these small molecules in this respect, including for the treatment of PMM2-CDG.

**Keywords:** Conformational diseases; congenital disorders of glycosylation; inborn errors of metabolism; misfolding diseases; pharmacological chaperones; protein folding; proteostasis regulators.

---

## Introduction

Proteostasis requires that a delicate balance be maintained between protein synthesis, folding, trafficking and degradation. The cell is thus equipped with protein quality control systems (QCS) in which chaperone molecules, the ubiquitin proteasome pathway, and autophagy play important roles (1).

In eukaryotic cells, protein folding occurs in a number of cell compartments, including the mitochondria and peroxisomes, the much more massive endoplasmic reticulum (ER) (involved in the folding of membrane and secreted proteins), the cytosol, and the nucleus. Cells respond to protein folding problems following different strategies depending on the compartment in which they arise: the unfolded protein response (UPR) in the ER, the mitochondrial UPR in the mitochondria, and the heat-shock response (HSR) in the nucleus and cytosol (2, 3). These responses are made to small perturbations in protein homeostasis and play vital roles in helping misfolded proteins regain their correct conformation (4).

Molecular chaperones are central elements of protein QCSs. They interact co-translationally with nascent polypeptide chains, helping non-native proteins acquire a native conformation (5). A number of conserved families of molecular chaperones guide cytosolic proteins in efforts to prevent misfolding and aggregation. Their members are referred to as stress proteins or heat shock proteins (Hsps) and are produced in larger amounts under conditions of conformational stress. The major chaperone families are classified by molecular weight (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100, and the small Hsp) (6). Heat shock factor 1 (HSF1) is a key molecule in the coordination of the HSR. This transcription factor is activated during cellular stress induced by the presence of unfolded proteins, leading to the transcription of chaperones and other molecules that modulate folding.

Protein misfolding induced by missense mutations has been identified as the cause of many genetic (or in this case conformational) diseases (7-9). Loss of protein function results from early degradation, mislocalization, structural alteration or aggregation (10-13) leading to pathological dysfunctions (14). When misfolded proteins cannot be properly refolded, the ubiquitin-proteasome system, autophagy and ER-associated degradation begin to degrade them (15-17).

Knowledge of the proteostasis network and protein QCS is, however, paving the way for new treatment strategies for protein misfolding diseases. Influencing the proteostasis network, or directly stabilizing target proteins using proteostasis regulators (PRs) or pharmacological chaperones (PCs), offers the possibility of treating several severe diseases (12, 18).

## **New therapies for treating protein misfolding diseases**

PRs and PCs lie at the centre of a revolutionary approach for treating protein misfolding diseases. Their stabilizing effect on mutant proteins allows their correct delivery and activity. The degree of functional correction achieved depends on the absolute amount of protein rescued, its intrinsic activity, and its new stability at the appropriate functional location.

*Proteostasis regulators.* PRs improve proteostasis by facilitating protein folding, enhancing the degradation of non-native protein species, and minimizing misfolding. This is achieved by increasing the function and availability of molecular chaperones, and/or the activation of the protein QCS (12). Small molecule PRs, such as non-steroidal anti-inflammatory drugs (19), proteasome inhibitors (20-22), celastrol (23, 24), Hsp90 inhibitors (25-27) and the benzyl pyrazole derivative HSF1A (28), are able to modulate the HSR. Other small molecule PRs, such as rapamycin (29), inositol-lowering compounds (30), and the inhibitor of USP14 (31) modulate autophagy or the ubiquitin proteasome system. The capacity of the proteostasis network can be enhanced by the modulation of calcium signalling (32) via the use of thapsigargin (33) or curcumin (34), among others (35).

The modulation of UPR and ER-associated degradation is mediated by different stress-response transmembrane proteins (36), including transcription factor 6 (ATF6), protein kinase RNA (PKR)-like ER kinase (PERK), and inositol-requiring protein 1 (IRE1). These integral membrane proteins act as ER stress transducers and respond to the accumulation of misfolded proteins in the ER lumen by leading to the activation of transcription factors that regulate the expression of UPR target genes (35).

*Pharmacological chaperones.* PCs bind to proteins via electrostatic forces, van der Waals forces, or hydrogen bonding. They do not have the ability to refold the target protein, but they are able to shift the equilibrium toward the folded state and, therefore, are valid for pathogenic mutations that induce protein denaturation. Specific ligand-binding sites are often located at the interface between protein domains or subdomains; the corresponding ligands can therefore be particularly effective at stabilizing a protein's structure (37). PCs are protein specific, and some are mutation specific.

The most commonly used PCs directed against misfolded enzymes are based on their substrates, though these suffer from the drawback of inhibiting enzyme function when in high concentration (38). However, new, non-substrate-like and therefore non-inhibitory PCs have been used in the treatment of certain genetic disorders (39). The emerging concepts of PC therapy include both inhibitory and non-inhibitory (allosteric) strategies. The former involve competitive PCs that reversibly bind to the active site of the target enzyme via hydrogen bond networks and van der Waal's interactions. These have been used in the treatment of lysosomal diseases to help form stable protein complexes in the ER that are then transported to the lysosome. Here, these enzymes remain stable but are catalytically active for some time (40). In allosteric PC therapy, however, the chaperone is non-competitive and binds to a site other than the active site (as in methylmalonic aciduria *cblB* type) (41). For example, tafadamis is an allosteric PC that has received market approval for transthyretin-related hereditary amyloidosis (42, 43). These PCs do not inhibit the target enzyme and thus have the greatest potential as the next generation of chaperones, either alone or in combination with PCs that target the active site (44). Enzyme cofactors provide another type of PC. An increase in the amount of the natural cofactor of an enzyme might help stabilize it (45). Such is the case of (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH<sub>4</sub>), the natural cofactor of phenylalanine hydroxylase (PAH), the defective enzyme in phenylketonuria (PKU) (46, 47). Additionally, endogenous Hsp can be used for the stabilization of mutant proteins in some diseases by using molecular chaperone inducers (6, 48).

PCs hold the promise of being able to reduce the severity of several genetic diseases, but it is important to note that the number of PCs described for different targets is low. For instance, in cystic fibrosis (CF), a number of PCs and PRs that might rescue CF transmembrane conductance regulator (CFTR) have recently been identified (34, 49-51), one of which is the PR roscovitine, which is involved in calcium regulation (52). The use of therapeutic autophagy inducers has also been tested in CF. For example, the repurposed drug cysteamine - which is FDA-approved for treating cystinosis (53) - has been shown to restore CFTR function in a clinical trial involving CF patients (54).

When a PC therapy is mutation-specific it cannot, of course, be prescribed for all patients with the same genetic disease (48). However, some PCs are *not* mutation-specific. Many mutant forms of gonadotropin releasing hormone receptor, vasopressin

type 2 receptor, and rhodopsin have been successfully rescued in cell cultures by the same PCs (55), offering hope that a single PC could be used to treat patients with different mutant forms of the target enzyme.

PRs could also be used in combination with PCs (56). PCs stabilise the pool of natively folded proteins, while PRs increase the proteostasis network capacity. Their co-administration might therefore have additive or synergistic effects (2, 18). PRs activate the UPR, which increases the pool of folded mutant proteins which PCs can then stabilize. The elucidation of the exact mechanism by which the protein QCS acts in specific diseases, might allow the design of improved treatment options for individual patients (57).

### **Protein misfolding diseases**

The first step before attempting any type of pharmacotherapy is to determine whether the disease under study might be responsive to this type of treatment. A candidate disease for folding therapy is one that involves destabilizing missense mutations leading to the unstable proteins formed being degraded or aggregated. The gathering of computational evidence, such as that afforded by FoldX (58) or residue location analysis, together with experimental evidence regarding the oligomeric state of the mutant protein and its stability (59), are necessary steps in determining whether a missense variation alters a protein's folding. The fact that PRs act within the protein homeostasis network rather than directly on any specific protein may render them valuable against a whole group of diseases that alter a particular process in a similar fashion (45).

#### *Neurodegenerative diseases caused by misfolded functional proteins*

The formation of insoluble oligomers and toxic aggregates of misfolded functional proteins can lead to a toxic gain-of-function. The accumulation of toxic aggregates in the brain is an upstream event in the pathological cascade leading to neuronal dysfunction, cell death, and eventually neurodegenerative diseases such as Alzheimer, Parkinson, Huntington and Creutzfeldt-Jakob disease (60, 61). Amyloid plaques and neurofibrillary tangles are the hallmarks of Alzheimer disease, and both arise from protein misfolding (62); the A $\beta$  peptide and tau protein suffer conformational changes

that produce toxic aggregates (63). In Parkinson disease, the death of dopaminergic neurons and protein aggregation (Lewy bodies) is present (64). The use of PCs/PRs that prevent or reduce neurodegeneration in Parkinson disease models and related synucleinopathies have been described. Those most commonly used in Parkinson disease are geldanamycin, celastrol, trehalose and 4-phenylbutyrate. The most frequently used PRs in synucleinopathies are HSP90 inhibitors (geldanamycin, 17-AAG, SNX compounds), and enhancers of HSF-1 (carbenoxolone). Chemical chaperones such as trehalose and mannitol may also have a clinical future (42, 65).

#### *Dominant diseases caused by misfolded structural proteins*

Misfolded structural proteins that are not degraded may also form toxic aggregates in the cell. Monogenic conformational diseases with a dominant inheritance pattern include familial cardiomyopathies (66), collagen disorders (67) and keratin disorders (68). In epidermolysis bullosa simplex, an inherited connective tissue disorder, mutant forms of the keratin proteins KRT5 and KRT14 lead to severe blistering of the skin in response to injury (14). Disease-associated mutations in the keratin gene cause the misfolding and aggregation of the protein, particularly in response to mechanical stress (69, 70). Depending on the nature and position of the causal mutation, these diseases may be mild or severe. Inherited Li-Fraumeni syndrome and some early onset cancers involving p53 mutations (71) may be added to this negative dominant type of protein misfolding diseases (72).

#### *Recessive metabolic genetic diseases*

The term 'inborn errors of metabolism' (IEM) covers a large group of conditions affecting the biosynthesis or breakdown of biomolecules involved in specific pathways, recognizable by specific biochemical tests. Recently, it has become necessary to redefine IEMs since they have been found not only to involve metabolic pathways but also cellular processes (73). Most IEMs remain without effective treatment. However, in recent years, the therapeutic use of small molecules has emerged as a promising possibility in the treatment of protein folding disease - the mechanism behind many autosomal recessive metabolic disorders. The resulting pathologies may involve misfolded proteins in the cytosol, e.g., PKU (74), mitochondrial methylmalonic aciduria *cblB* type (13), or organelles, e.g., lysosomal storage disorders (LSDs). The degree of

the misfolding determines the severity and outcome of the associated disease; a range of phenotypes are therefore usually observed (12, 72).

### Conformational disorders in the cytosol compartment

PKU, the first well-known misfolding disease (38), is caused by mutations affecting the PAH enzyme, which is responsible for metabolizing the essential amino acid phenylalanine. Most disease-causing mutations in the phenylalanine hydroxylase gene produce misfolded proteins that are rapidly degraded. A considerable subset of patients respond to treatment with the protein's natural cofactor BH<sub>4</sub> which, in certain mutations, can act as a PC (46, 75). The approval of sapropterin dihydrochloride as an orphan drug for BH<sub>4</sub>-responsive PAH deficiency (76-78), which in effect saw an oral pharmacological treatment substitute or combine with a traditional dietary treatment, marked a paradigm shift in the treatment of this disease. Nowadays, approximately half of all PKU patients benefit from this therapy. Efforts are being focused on the discovery of cofactor analogues with improved pharmacokinetic properties, as well as non-substrate-like analogues (79). Candidate PCs have also been identified through shape-focused virtual screening (80).

Classical homocystinuria is caused by mutations affecting the cystathionine  $\beta$ -synthase (CBS) protein. Since 85% of these are of the missense type, it has been postulated that protein misfolding plays an important role in the pathogenesis of CBS deficiency (81). An important fraction of patients responds to pharmacological doses of pyridoxine, increasing hepatic CBS activity (82). Heme arginate also increases CBS residual activity and promotes proper enzyme assembly *in vitro*; although the clinical use of heme arginate may be limited by its cost and potential side-effects (81). Recently, the first evidence has been reported for the use of S-adenosylmethionine as a kinetic stabilizer for CBS (83, 84), along with different molecules acting as chemical chaperones, such as betaine and taurine (85), DMSO, glycerol, proline and TMAO (86). PRs have also been used to rescue destabilizing CBS mutants; the proteasome inhibitors, bortezomib and ONX0912 have both been proven effective in an animal model of homocystinuria (87). In primary hyperoxaluria type 1, a disorder of glyoxylate metabolism, betaine and pyridoxine have been suggested as PCs (88).



The authors' group is investigating a range of molecular therapies for congenital disorder of glycosylation (CDG) due to phosphomannomutase 2 deficiency (PMM2-CDG or CDG type Ia) (59, 89). PMM2 is a homodimeric enzyme that catalyzes the conversion of mannose-1-phosphate into mannose-6-phosphate (90). This metabolic disease involves a defect in protein glycosylation, for which no effective treatment is available. PMM2-CDG is the most common form of CDG.

Loss-of-function mutations in patients with PMM2-CDG involve the increased susceptibility of PMM2 to degradation and/or aggregation. In certain cases, however, including the mutations characterized by our group, i.e., p.L32R, p.V44A, p.D65Y, p.P113L, p.R123Q, p.R141H, p.F157S, p.R162W, p.F183S, p.P184T, p.F207S, p.T237M and p.C241S (91, 92), PMM2 activity might be rescuable via the use of synergetic PRs and/or PCs. The fact that 80% of PMM2 mutations are missense (HGMD professional release), and that most have been identified in a compound heterozygous state -with one severe null mutation and one destabilizing mutation allowing PMM2 to retain some residual activity (89, 93)- suggests that PMM2-CDG is a conformational disease (59) that PCs might be able to alleviate in many patients. In our work, eight possible PCs were selected from a 10,000 compound library screening. The compound 1-(3-chlorophenyl)-3-3-bis(pyridine-2-yl)urea (compound VIII) stood out, based on its pharmacochemical properties, its lack of an inhibitory effect on PMM2 activity, and its positive effect on the activity and stability of a number of destabilizing PMM2 mutations. Together, the results provided the first proof-of-concept that PCs can be used to treat PMM2-CDG, plus a basis for developing a new therapy based on the chemical optimization of compound VIII (89). We are currently exploring the effect of PRs on the activity and amount of protein produced by different PMM2-CDG mutants. The next step will be to study whether the PC compound VIII and PRs have a synergistic effect.

### Mitochondrial conformational disorders

A PC for the treatment of methylmalonic aciduria cblB type was also identified by our group via the high-throughput ligand screening of more than 2000 compounds. One of these compounds, N{[4-chlorophenyl]carbamothioyl]amino}-2-phenylacetamide, was selected based on the increase it afforded in the stability of purified ATP:cob(I)alamin adenosyltransferase (ATR). It also enhanced ATR activity in fibroblasts from patient

with a destabilizing hemizygous I96T mutation. Cobalamin, when present, improved its effect. An increase in steady-state levels of the ATR protein was also observed in both the liver and brain of mice after 12 days of oral administration of the compound (41). Our group has now characterized a number of destabilizing mutations that respond to treatment with this compound (data not published).

It is also reported that, in maple syrup urine disease (MSUD) (94) and pyruvate dehydrogenase (PDH) deficiency, the small molecule phenylbutyrate acts by increasing the residual activity of enzymes via the inhibition of their inactivating enzymes (94, 95).

Certainly, protein misfolding is a hallmark of fatty acid oxidation defects, and small molecules able to increase the functional level of the affected protein are valuable candidates in drug design. Mitochondrial cofactors and metabolites have been identified as potential stabilizers of two  $\beta$ -oxidation acyl-CoA dehydrogenases - short chain acyl-CoA dehydrogenase and the medium chain acyl-CoA dehydrogenase -as well as of glutaryl-CoA dehydrogenase, an enzyme involved in lysine and tryptophan metabolism. It is also reported that physiological concentrations of FAD result in a spectacular improvement in the thermal stabilities of these enzymes, and preventing their loss of activity (96). Riboflavin (vitamin B<sub>2</sub>) has also been reported a potential therapeutic agent for disorders affecting mitochondrial energy metabolism (97).

### Lysosomal storage disorders

Most LSDs are caused by the malfunction of one of the lysosome hydrolases responsible for the catabolism of glycogen, peptides, glycoproteins, mucopolysaccharides, oligosaccharides or cholesterol, leading to pathological substrate accumulation (98). LSDs represent a pharmacological therapy success story; those now treated with PCs include Fabry, Pompe and Gaucher disease, GM1-gangliosidosis (Tay-Sachs disease), GM2-gangliosidosis (Sandhoff disease), Krabbe disease, Batten disease, and Sanfilippo syndrome type C. Most of the PCs used are substrate analogues (38). The breakthrough in this kind of therapy was first reported in Fabry disease, for which a small-molecule inhibitor was found that selectively binds to and stabilizes the target protein (99). The first substrate inhibitor that received market approval was Miglustat - for Gaucher disease type I in 2002 (100), and for Niemann-Pick disease type C in 2009 (101). Miglustat, a glucosylceramide synthase inhibitor, prevents the formation of the substrate that would normally build up in the presence of an enzyme deficient in its

breakdown. It is used to treat mild-to-moderate type I Gaucher disease when enzyme replacement therapy does not return the hoped-for results. In the first clinical trial with Miglustat, a significant reduction in disease biomarkers was reported (102). Another substrate inhibitor, Eliglustat was granted marketing authorization in 2015. This is also a glucosylceramide synthase inhibitor, but more specific and potent than Miglustat, and represents an emerging alternative to enzyme replacement therapy for the long-term treatment of adults with Gaucher disease type I (103, 104). It is important to note that Miglustat has poor central nervous system penetration. New substrate inhibitors able to cross the brain blood barrier have, however, been developed and have returned promising results in Gaucher and Fabry disease (105). Since it is possible that all diseases caused by a deficiency in one enzyme involved in the same degradation pathway might be responsive to the same substrate inhibitor, Miglustat has also been tested in Tay-Sachs (106) and Sandhoff (107) patients. However, the results obtained have been controversial.

Additionally, the proteasome inhibitor bortezomib has been described to significantly improve the activity of Niemann-Pick type C mutant proteins caused by specific missense variants (108), and in Gaucher disease the proteasome inhibitor MG132, together with the heat-shock transcription factor 1 activator celastrol, have been shown to enhance the folding, trafficking and activity of misfolded glucosylceramidase proteins caused by different missense mutations (18). In Gaucher disease, calcium regulation with lacidipine is also being investigated with promising results (109), and TRMPL1 ligands are showing promise in mucopolipidosis type IV (110). A curcumin derivative, BCM95, along with an autophagy inducer, hydroxypropyl- $\beta$ -cyclodextrin, may also be effective in the treatment of saposin C deficiency via the improvement of lysosome function (111). Finally, autophagy inducers have been proven of potential use via the treatment of cystinosis with cysteamine (112).

In total, around 40 PCs have been described as effective in the treatment of different IEMs, most of them for LSDs (12, 45), but of these only few are used clinically or are under pharmaceutical development (Table 1).

### **Proteostasis regulators and pharmacological chaperones in the clinic**

The first step in developing pharmacotherapies involves the identification of potential therapeutic molecules. Two strategies can be followed: high-throughput screenings

(HTSs), exploring libraries of thousands of existing chemicals and drugs, or hypothesis-driven searching. By monitoring thermal protein stability using differential scanning fluorimetry, HTS are particularly useful for identifying novel chemical structures (79). Hypothesis-driven strategies are usually based on screening for low molecular weight compounds structurally related to key moieties of the natural substrate, its cofactors or inhibitors (12, 113).

The development of PRs or PCs by virtual screening is based on computational investigations. This has the major advantage of allowing large numbers of compounds to be investigated at the same time, increasing the probability of finding hits and improving the likelihood of being able to select allosteric binding sites. One of the most used virtual screening techniques is molecular docking. Another strategy is the development of a pharmacophore model - an ensemble of steric and electronic features that ensure optimal supramolecular interactions with a specific biological target (79). Multicomponent drug discovery can also be undertaken using computational tools to perform repositioning analysis. *In silico* predictions have been shown powerful enough to lead to new and efficient potential therapies, e.g., for amyotrophic lateral sclerosis (114).

When a hit is selected, it needs to pass a variety of experimental and computational filters to ensure its viability as a therapeutic agent. Once preclinical assays succeed, the most promising candidate for a given disease is selected, and is then used in animal experiments prior to entering clinical trials with human patients. The last stage of the drug discovery process is the derivatization of ligands. Lead optimization improves some of the properties of molecules, such as lipophilicity, synthetic accessibility, absorption, distribution, metabolism, toxicity and excretion (115). It should be noted that many hit compounds do not make it to the clinical stage of development since they are, in fact, artefacts, i.e., their activity does not depend on a specific, drug-like interaction with the target protein. Such molecules are termed pan-assay interference compounds (PAINS) (116). To avoid the problems caused by PAINS, the selection and characterization methods followed during the first steps of drug discovery need to be improved (117). Table 1 shows the diseases for which molecular therapies are under clinical trials in humans or are already on the market.

## Perspectives

The discovery of PCs can be accelerated by contemplating drug repositioning. Several small molecule drugs have been successfully repositioned as PCs for rare disorders, including doxorubicin, an anti-neoplastic anthracycline now used in CF; diltiazem, an antihypertensive now used in Gaucher disease; ambroxol, a mucolytic agent now used for Gaucher and Fabry disease; acetylcysteine, another mucolytic agent now used in Pompe disease; pyrimethamine, an anti-parasitic compound now used in GM2 gangliosidosis; carbamazepine, a dibenzazepine now used in hyperinsulinaemic hypoglycaemia; and salicylate which has found a use in the treatment of Pendred syndrome (118).

Combining PCs and PRs will likely prove the most effective treatments for protein misfolding diseases, one molecule acting as a stress-responsive signalling pathway activator, the other binding to and stabilizing the misfolded protein (56).

## **Acknowledgments**

This work was funded by the *Fondo Investigación Sanitaria*, grants PI13/01239 and PI16/00573; the *Fundación Isabel Gemio*, an institutional grant from the *Fundación Ramón Areces* and by the European Regional Development Fund. The authors thank all the PMM2-CDG families involved in the research undertaken at our laboratory.

## **Abbreviations**

ATR: ATP:cob(I)alamin adenosyltransferase

BH<sub>4</sub>: (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin

CBS: Cystathionine  $\beta$ -synthase

CDG: Congenital disorders of glycosylation

CF: Cystic fibrosis

CFTR: Cystic fibrosis transmembrane conductance regulator

ER: Endoplasmic reticulum

Hsp: Heat-shock proteins

HSR: Heat-shock response

HTS: High-throughput screening

IEM: Inborn error of metabolism

LSD: Lysosomal storage disorders

PAH: Phenylalanine hydroxylase

PAINS: Pan-assay interference compounds

PC: Pharmacological chaperone

PKU: Phenylketonuria

PMM2: Phosphomannomutase 2

PR: Proteostasis regulator

QCS: Quality control system

UPR: Unfolded protein response

## **Figure legends**

### **Figure 1. Drug discovery for PMM2-CDG**

The drug discovery process starts with the functional characterization of PMM2-CDG causing mutations identified in patients. Then an initial high throughput screening of 10,000 compounds from a commercial library allows the selection of molecules that act as potential pharmacological chaperones (PCs) for the PMM2 protein; then the validation of these PCs in different systems, as well as by computational analysis (<http://pasilla.health.unm.edu/tomcat/biocomp/smartsfilter>), leads to the optimization of the selected hit-compound.

## References

1. Balch WE, Morimoto RI, Dillin A et al. Adapting proteostasis for disease intervention. *Science* 2008; 319: 916-919.
2. Wang YJ, Di XJ, Mu TW. Using pharmacological chaperones to restore proteostasis. *Pharmacol Res* 2014; 83: 3-9.
3. Chambers JE, Marciniak SJ. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 2. Protein misfolding and ER stress. *Am J Physiol Cell Physiol* 2014; 307: C657-670.
4. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature* 2011; 475: 324-332.
5. Hartl FU. Molecular chaperones in cellular protein folding. *Nature* 1996; 381: 571-579.
6. Balchin D, Hayer-Hartl M, Hartl FU. In vivo aspects of protein folding and quality control. *Science* 2016; 353: aac4354.
7. Martinez A, Calvo AC, Teigen K et al. Rescuing proteins of low kinetic stability by chaperones and natural ligands phenylketonuria, a case study. *Prog Mol Biol Transl Sci* 2008; 83: 89-134.
8. Carrell RW, Lomas DA. Conformational disease. *Lancet* 1997; 350: 134-138.
9. Beissinger M, Buchner J. How chaperones fold proteins. *Biol Chem* 1998; 379: 245-259.
10. Ulloa-Aguirre A, Janovick JA, Brothers SP et al. Pharmacologic rescue of conformationally-defective proteins: implications for the treatment of human disease. *Traffic* 2004; 5: 821-837.
11. Gregersen N. Protein misfolding disorders: pathogenesis and intervention. *J Inherit Metab Dis* 2006; 29: 456-470.
12. Muntau AC, Leandro J, Staudigl M et al. Innovative strategies to treat protein misfolding in inborn errors of metabolism: pharmacological chaperones and proteostasis regulators. *J Inherit Metab Dis* 2014; 37: 505-523.
13. Jorge-Finnigan A, Aguado C, Sanchez-Alcudia R et al. Functional and structural analysis of five mutations identified in methylmalonic aciduria cblB type. *Hum Mutat* 2010; 31: 1033-1042.
14. Valastyan JS, Lindquist S. Mechanisms of protein-folding diseases at a glance. *Dis Model Mech* 2014; 7: 9-14.
15. Smith MH, Ploegh HL, Weissman JS. Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. *Science* 2011; 334: 1086-1090.
16. Varshavsky A. The ubiquitin system, an immense realm. *Annu Rev Biochem* 2012; 81: 167-176.
17. Fredrickson EK, Gardner RG. Selective destruction of abnormal proteins by ubiquitin-mediated protein quality control degradation. *Semin Cell Dev Biol* 2012; 23: 530-537.
18. Mu TW, Ong DS, Wang YJ et al. Chemical and biological approaches synergize to ameliorate protein-folding diseases. *Cell* 2008; 134: 769-781.
19. Lee BS, Chen J, Angelidis C et al. Pharmacological modulation of heat shock factor 1 by antiinflammatory drugs results in protection against stress-induced cellular damage. *Proc Natl Acad Sci U S A* 1995; 92: 7207-7211.
20. Westerheide SD, Morimoto RI. Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J Biol Chem* 2005; 280: 33097-33100.

21. Neef DW, Jaeger AM, Thiele DJ. Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. *Nat Rev Drug Discov* 2011; 10: 930-944.
22. Dick LR, Fleming PE. Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy. *Drug Discov Today* 2010; 15: 243-249.
23. Westerheide SD, Bosman JD, Mbadugha BN et al. Celastrols as inducers of the heat shock response and cytoprotection. *J Biol Chem* 2004; 279: 56053-56060.
24. Trott A, West JD, Klaic L et al. Activation of heat shock and antioxidant responses by the natural product celastrol: transcriptional signatures of a thiol-targeted molecule. *Mol Biol Cell* 2008; 19: 1104-1112.
25. Zou J, Guo Y, Guettouche T et al. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* 1998; 94: 471-480.
26. Vaughan CK, Neckers L, Piper PW. Understanding of the Hsp90 molecular chaperone reaches new heights. *Nat Struct Mol Biol* 2010; 17: 1400-1404.
27. Ansar S, Burlison JA, Hadden MK et al. A non-toxic Hsp90 inhibitor protects neurons from Abeta-induced toxicity. *Bioorg Med Chem Lett* 2007; 17: 1984-1990.
28. Neef DW, Turski ML, Thiele DJ. Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. *PLoS Biol* 2010; 8: e1000291.
29. Sarkar S, Perlstein EO, Imarisio S et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat Chem Biol* 2007; 3: 331-338.
30. Sarkar S, Rubinsztein DC. Small molecule enhancers of autophagy for neurodegenerative diseases. *Mol Biosyst* 2008; 4: 895-901.
31. Lee BH, Lee MJ, Park S et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* 2010; 467: 179-184.
32. Krebs J, Agellon LB, Michalak M. Ca(2+) homeostasis and endoplasmic reticulum (ER) stress: An integrated view of calcium signaling. *Biochem Biophys Res Commun* 2015; 460: 114-121.
33. Egan ME, Glockner-Pagel J, Ambrose C et al. Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. *Nat Med* 2002; 8: 485-492.
34. Egan ME, Pearson M, Weiner SA et al. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004; 304: 600-602.
35. Calamini B, Morimoto RI. Protein homeostasis as a therapeutic target for diseases of protein conformation. *Curr Top Med Chem* 2012; 12: 2623-2640.
36. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; 8: 519-529.
37. Ringe D, Petsko GA. What are pharmacological chaperones and why are they interesting? *J Biol* 2009; 8: 80.
38. Aymami J, Barril X, Rodriguez-Pascau L et al. Pharmacological chaperones for enzyme enhancement therapy in genetic diseases. *Pharm Pat Anal* 2013; 2: 109-124.
39. Leidenheimer NJ, Ryder KG. Pharmacological chaperoning: a primer on mechanism and pharmacology. *Pharmacol Res* 2014; 83: 10-19.
40. Tropak MB, Blanchard JE, Withers SG et al. High-throughput screening for human lysosomal beta-N-Acetyl hexosaminidase inhibitors acting as pharmacological chaperones. *Chem Biol* 2007; 14: 153-164.
41. Jorge-Finnigan A, Brasil S, Underhaug J et al. Pharmacological chaperones as a potential therapeutic option in methylmalonic aciduria cblB type. *Hum Mol Genet* 2013; 22: 3680-3689.



42. Bulawa CE, Connelly S, Devit M et al. Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci U S A* 2012; 109: 9629-9634.
43. Maurer MS, Grogan DR, Judge DP et al. Tafamidis in transthyretin amyloid cardiomyopathy: effects on transthyretin stabilization and clinical outcomes. *Circ Heart Fail* 2015; 8: 519-526.
44. Porto C, Ferrara MC, Meli M et al. Pharmacological enhancement of alpha-glucosidase by the allosteric chaperone N-acetylcysteine. *Mol Ther* 2012; 20: 2201-2211.
45. Matalonga L, Gort L, Ribes A. Small molecules as therapeutic agents for inborn errors of metabolism. *J Inherit Metab Dis* 2017; 40: 177-193.
46. Pey AL, Perez B, Desviat LR et al. Mechanisms underlying responsiveness to tetrahydrobiopterin in mild phenylketonuria mutations. *Hum Mutat* 2004; 24: 388-399.
47. Staudigl M, Gersting SW, Danecka MK et al. The interplay between genotype, metabolic state and cofactor treatment governs phenylalanine hydroxylase function and drug response. *Hum Mol Genet* 2011; 20: 2628-2641.
48. Suzuki Y. Emerging novel concept of chaperone therapies for protein misfolding diseases. *Proc Jpn Acad Ser B Phys Biol Sci* 2014; 90: 145-162.
49. Denny RA, Gavrin LK, Saiah E. Recent developments in targeting protein misfolding diseases. *Bioorg Med Chem Lett* 2013; 23: 1935-1944.
50. Esposito S, Tosco A, Villella VR et al. Manipulating proteostasis to repair the F508del-CFTR defect in cystic fibrosis. *Mol Cell Pediatr* 2016; 3: 13.
51. Dey I, Shah K, Bradbury NA. Natural Compounds as Therapeutic Agents in the Treatment Cystic Fibrosis. *J Genet Syndr Gene Ther* 2016; 7.
52. Norez C, Vandebrouck C, Bertrand J et al. Roscovitine is a proteostasis regulator that corrects the trafficking defect of F508del-CFTR by a CDK-independent mechanism. *Br J Pharmacol* 2014; 171: 4831-4849.
53. Langman CB, Greenbaum LA, Sarwal M et al. A randomized controlled crossover trial with delayed-release cysteamine bitartrate in nephropathic cystinosis: effectiveness on white blood cell cystine levels and comparison of safety. *Clin J Am Soc Nephrol* 2012; 7: 1112-1120.
54. Charrier C, Rodger C, Robertson J et al. Cysteamine (Lynovex(R)), a novel mucoactive antimicrobial & antibiofilm agent for the treatment of cystic fibrosis. *Orphanet J Rare Dis* 2014; 9: 189.
55. Conn PM, Janovick JA. Drug development and the cellular quality control system. *Trends Pharmacol Sci* 2009; 30: 228-233.
56. Lindquist SL, Kelly JW. Chemical and biological approaches for adapting proteostasis to ameliorate protein misfolding and aggregation diseases: progress and prognosis. *Cold Spring Harb Perspect Biol* 2011; 3.
57. Gregersen N, Bross P, Jorgensen MM et al. Defective folding and rapid degradation of mutant proteins is a common disease mechanism in genetic disorders. *J Inherit Metab Dis* 2000; 23: 441-447.
58. Schymkowitz J, Borg J, Stricher F et al. The FoldX web server: an online force field. *Nucleic Acids Res* 2005; 33: W382-388.
59. Yuste-Checa P, Gamez A, Brasil S et al. The Effects of PMM2-CDG-Causing Mutations on the Folding, Activity, and Stability of the PMM2 Protein. *Hum Mutat* 2015; 36: 851-860.
60. Huang A, Stultz CM. Finding order within disorder: elucidating the structure of proteins associated with neurodegenerative disease. *Future Med Chem* 2009; 1: 467-482.

61. Cortez L, Sim V. The therapeutic potential of chemical chaperones in protein folding diseases. *Prion* 2014: 8.
62. Hetz C, Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. *Nat Rev Neurosci* 2014; 15: 233-249.
63. Diaz-Villanueva JF, Diaz-Molina R, Garcia-Gonzalez V. Protein Folding and Mechanisms of Proteostasis. *Int J Mol Sci* 2015; 16: 17193-17230.
64. Van Laar VS, Arnold B, Cassady SJ et al. Bioenergetics of neurons inhibit the translocation response of Parkin following rapid mitochondrial depolarization. *Hum Mol Genet* 2011; 20: 927-940.
65. Ebrahimi-Fakhari D, Saidi LJ, Wahlster L. Molecular chaperones and protein folding as therapeutic targets in Parkinson's disease and other synucleinopathies. *Acta Neuropathol Commun* 2013; 1: 79.
66. Burch M, Blair E. The inheritance of hypertrophic cardiomyopathy. *Pediatr Cardiol* 1999; 20: 313-316.
67. Baum J, Brodsky B. Folding of peptide models of collagen and misfolding in disease. *Curr Opin Struct Biol* 1999; 9: 122-128.
68. Sorensen CB, Ladekjaer-Mikkelsen AS, Andresen BS et al. Identification of novel and known mutations in the genes for keratin 5 and 14 in Danish patients with epidermolysis bullosa simplex: correlation between genotype and phenotype. *J Invest Dermatol* 1999; 112: 184-190.
69. Werner NS, Windoffer R, Strnad P et al. Epidermolysis bullosa simplex-type mutations alter the dynamics of the keratin cytoskeleton and reveal a contribution of actin to the transport of keratin subunits. *Mol Biol Cell* 2004; 15: 990-1002.
70. Russell D, Andrews PD, James J et al. Mechanical stress induces profound remodelling of keratin filaments and cell junctions in epidermolysis bullosa simplex keratinocytes. *J Cell Sci* 2004; 117: 5233-5243.
71. Monti P, Campomenosi P, Ciribilli Y et al. Tumour p53 mutations exhibit promoter selective dominance over wild type p53. *Oncogene* 2002; 21: 1641-1648.
72. Gregersen N, Bolund L, Bross P. Protein misfolding, aggregation, and degradation in disease. *Mol Biotechnol* 2005; 31: 141-150.
73. Morava E, Rahman S, Peters V et al. Quo vadis: the re-definition of "inborn metabolic diseases". *J Inherit Metab Dis* 2015; 38: 1003-1006.
74. Pey AL, Desviat LR, Gamez A et al. Phenylketonuria: genotype-phenotype correlations based on expression analysis of structural and functional mutations in PAH. *Hum Mutat* 2003; 21: 370-378.
75. Sarkissian CN, Gamez A, Scott P et al. Chaperone-like therapy with tetrahydrobiopterin in clinical trials for phenylketonuria: is genotype a predictor of response? *JIMD Rep* 2012; 5: 59-70.
76. Levy HL, Milanowski A, Chakrapani A et al. Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study. *Lancet* 2007; 370: 504-510.
77. Lee P, Treacy EP, Crombez E et al. Safety and efficacy of 22 weeks of treatment with sapropterin dihydrochloride in patients with phenylketonuria. *Am J Med Genet A* 2008; 146A: 2851-2859.
78. Trefz FK, Burton BK, Longo N et al. Efficacy of sapropterin dihydrochloride in increasing phenylalanine tolerance in children with phenylketonuria: a phase III, randomized, double-blind, placebo-controlled study. *J Pediatr* 2009; 154: 700-707.

79. Pey AL, Ying M, Cremades N et al. Identification of pharmacological chaperones as potential therapeutic agents to treat phenylketonuria. *J Clin Invest* 2008; 118: 2858-2867.
80. Santos-Sierra S, Kirchmair J, Perna AM et al. Novel pharmacological chaperones that correct phenylketonuria in mice. *Hum Mol Genet* 2012; 21: 1877-1887.
81. Melenovska P, Kopecka J, Krijt J et al. Chaperone therapy for homocystinuria: the rescue of CBS mutations by heme arginate. *J Inherit Metab Dis* 2015; 38: 287-294.
82. Pey AL, Albert A, Salido E. Protein homeostasis defects of alanine-glyoxylate aminotransferase: new therapeutic strategies in primary hyperoxaluria type I. *Biomed Res Int* 2013; 2013: 687658.
83. Majtan T, Pey AL, Kraus JP. Kinetic stability of cystathionine beta-synthase can be modulated by structural analogs of S-adenosylmethionine: Potential approach to pharmacological chaperone therapy for homocystinuria. *Biochimie* 2016; 126: 6-13.
84. Pey AL, Majtan T, Sanchez-Ruiz JM et al. Human cystathionine beta-synthase (CBS) contains two classes of binding sites for S-adenosylmethionine (SAM): complex regulation of CBS activity and stability by SAM. *Biochem J* 2013; 449: 109-121.
85. Kopecka J, Krijt J, Rakova K et al. Restoring assembly and activity of cystathionine beta-synthase mutants by ligands and chemical chaperones. *J Inherit Metab Dis* 2011; 34: 39-48.
86. Majtan T, Liu L, Carpenter JF et al. Rescue of cystathionine beta-synthase (CBS) mutants with chemical chaperones: purification and characterization of eight CBS mutant enzymes. *J Biol Chem* 2010; 285: 15866-15873.
87. Gupta S, Wang L, Anderl J et al. Correction of cystathionine beta-synthase deficiency in mice by treatment with proteasome inhibitors. *Hum Mutat* 2013; 34: 1085-1093.
88. Fargue S, Rumsby G, Danpure CJ. Multiple mechanisms of action of pyridoxine in primary hyperoxaluria type 1. *Biochim Biophys Acta* 2013; 1832: 1776-1783.
89. Yuste-Checa P, Brasil S, Gamez A et al. Pharmacological Chaperoning: A Potential Treatment For PMM2-CDG. *Hum Mutat* 2016.
90. Matthijs G, Schollen E, Bjursell C et al. Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). *Hum Mutat* 2000; 16: 386-394.
91. Vega AI, Perez-Cerda C, Desviat LR et al. Functional analysis of three splicing mutations identified in the PMM2 gene: toward a new therapy for congenital disorder of glycosylation type Ia. *Hum Mutat* 2009; 30: 795-803.
92. Vega AI, Perez-Cerda C, Abia D et al. Expression analysis revealing destabilizing mutations in phosphomannomutase 2 deficiency (PMM2-CDG): expression analysis of PMM2-CDG mutations. *J Inherit Metab Dis* 2011; 34: 929-939.
93. Perez B, Briones P, Quelhas D et al. The molecular landscape of phosphomannose mutase deficiency in iberian peninsula: identification of 15 population-specific mutations. *JIMD Rep* 2011; 1: 117-123.
94. Brunetti-Pierri N, Lanpher B, Erez A et al. Phenylbutyrate therapy for maple syrup urine disease. *Hum Mol Genet* 2011; 20: 631-640.
95. Ferriero R, Manco G, Lamantea E et al. Phenylbutyrate therapy for pyruvate dehydrogenase complex deficiency and lactic acidosis. *Sci Transl Med* 2013; 5: 175ra131.
96. Lucas TG, Henriques BJ, Rodrigues JV et al. Cofactors and metabolites as potential stabilizers of mitochondrial acyl-CoA dehydrogenases. *Biochim Biophys Acta* 2011; 1812: 1658-1663.

97. Henriques BJ, Lucas TG, Gomes CM. Therapeutic Approaches Using Riboflavin in Mitochondrial Energy Metabolism Disorders. *Curr Drug Targets* 2016; 17: 1527-1534.
98. Valenzano KJ, Khanna R, Powe AC et al. Identification and characterization of pharmacological chaperones to correct enzyme deficiencies in lysosomal storage disorders. *Assay Drug Dev Technol* 2011; 9: 213-235.
99. Fan JQ, Ishii S, Asano N et al. Accelerated transport and maturation of lysosomal alpha-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. *Nat Med* 1999; 5: 112-115.
100. Aerts JM, Hollak CE, Boot RG et al. Substrate reduction therapy of glycosphingolipid storage disorders. *J Inherit Metab Dis* 2006; 29: 449-456.
101. Santos-Lozano A, Villamandos Garcia D, Sanchis-Gomar F et al. Niemann-Pick disease treatment: a systematic review of clinical trials. *Ann Transl Med* 2015; 3: 360.
102. Venier RE, Igdoura SA. Miglustat as a therapeutic agent: prospects and caveats. *J Med Genet* 2012; 49: 591-597.
103. Scott LJ. Eliglustat: A Review in Gaucher Disease Type 1. *Drugs* 2015; 75: 1669-1678.
104. Stirnemann J, Belmatoug N, Camou F et al. A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. *Int J Mol Sci* 2017; 18.
105. Marshall J, Sun Y, Bangari DS et al. CNS-accessible Inhibitor of Glucosylceramide Synthase for Substrate Reduction Therapy of Neuronopathic Gaucher Disease. *Mol Ther* 2016; 24: 1019-1029.
106. Shapiro BE, Pastores GM, Gianutsos J et al. Miglustat in late-onset Tay-Sachs disease: a 12-month, randomized, controlled clinical study with 24 months of extended treatment. *Genet Med* 2009; 11: 425-433.
107. Villamizar-Schiller IT, Pabon LA, Hufnagel SB et al. Neurological and cardiac responses after treatment with miglustat and a ketogenic diet in a patient with Sandhoff disease. *Eur J Med Genet* 2015; 58: 180-183.
108. Macias-Vidal J, Giros M, Guerrero M et al. The proteasome inhibitor bortezomib reduced cholesterol accumulation in fibroblasts from Niemann-Pick type C patients carrying missense mutations. *FEBS J* 2014; 281: 4450-4466.
109. Wang F, Chou A, Segatori L. Lacidipine remodels protein folding and Ca<sup>2+</sup> homeostasis in Gaucher's disease fibroblasts: a mechanism to rescue mutant glucocerebrosidase. *Chem Biol* 2011; 18: 766-776.
110. Chen CC, Keller M, Hess M et al. A small molecule restores function to TRPML1 mutant isoforms responsible for mucopolipidosis type IV. *Nat Commun* 2014; 5: 4681.
111. Tatti M, Motta M, Scarpa S et al. BCM-95 and (2-hydroxypropyl)-beta-cyclodextrin reverse autophagy dysfunction and deplete stored lipids in Sap C-deficient fibroblasts. *Hum Mol Genet* 2015; 24: 4198-4211.
112. Langman CB, Greenbaum LA, Grimm P et al. Quality of life is improved and kidney function preserved in patients with nephropathic cystinosis treated for 2 years with delayed-release cysteamine bitartrate. *J Pediatr* 2014; 165: 528-533 e521.
113. Calamini B, Silva MC, Madoux F et al. Small-molecule proteostasis regulators for protein conformational diseases. *Nat Chem Biol* 2011; 8: 185-196.
114. Herrando-Grabulosa M, Mulet R, Pujol A et al. Novel Neuroprotective Multicomponent Therapy for Amyotrophic Lateral Sclerosis Designed by Networked Systems. *PLoS One* 2016; 11: e0147626.

115. Issa NT, Wathieu H, Ojo A et al. Drug Metabolism in Preclinical Drug Development: A Survey of the Discovery Process, Toxicology, and Computational Tools. *Curr Drug Metab* 2017.
116. Whitty A. Growing PAINS in academic drug discovery. *Future Med Chem* 2011; 3: 797-801.
117. Baell J, Walters MA. Chemistry: Chemical con artists foil drug discovery. *Nature* 2014; 513: 481-483.
118. Hay Mele B, Citro V, Andreotti G et al. Drug repositioning can accelerate discovery of pharmacological chaperones. *Orphanet J Rare Dis* 2015; 10: 55.
119. Dawson G, Schroeder C, Dawson PE. Palmitoyl:protein thioesterase (PPT1) inhibitors can act as pharmacological chaperones in infantile Batten disease. *Biochem Biophys Res Commun* 2010; 395: 66-69.
120. Van Goor F, Hadida S, Grootenhuys PD et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc Natl Acad Sci U S A* 2011; 108: 18843-18848.
121. Benjamin ER, Khanna R, Schilling A et al. Co-administration with the pharmacological chaperone AT1001 increases recombinant human alpha-galactosidase A tissue uptake and improves substrate reduction in Fabry mice. *Mol Ther* 2012; 20: 717-726.
122. Motabar O, Liu K, Southall N et al. High throughput screening for inhibitors of alpha-galactosidase. *Curr Chem Genomics* 2010; 4: 67-73.
123. Motabar O, Sidransky E, Goldin E et al. Fabry disease - current treatment and new drug development. *Curr Chem Genomics* 2010; 4: 50-56.
124. Steet R, Chung S, Lee WS et al. Selective action of the iminosugar isofagomine, a pharmacological chaperone for mutant forms of acid-beta-glucosidase. *Biochem Pharmacol* 2007; 73: 1376-1383.
125. Rigat BA, Tropak MB, Buttner J et al. Evaluation of N-nonyl-deoxygalactonojirimycin as a pharmacological chaperone for human GM1 gangliosidosis leads to identification of a feline model suitable for testing enzyme enhancement therapy. *Mol Genet Metab* 2012; 107: 203-212.
126. Clarke JT, Mahuran DJ, Sathe S et al. An open-label Phase I/II clinical trial of pyrimethamine for the treatment of patients affected with chronic GM2 gangliosidosis (Tay-Sachs or Sandhoff variants). *Mol Genet Metab* 2011; 102: 6-12.
127. Berardi AS, Pannuzzo G, Graziano A et al. Pharmacological chaperones increase residual beta-galactocerebrosidase activity in fibroblasts from Krabbe patients. *Mol Genet Metab* 2014; 112: 294-301.
128. Sarkissian CN, Gamez A, Scott P et al. Chaperone-like therapy with tetrahydrobiopterin in clinical trials for phenylketonuria: is genotype a predictor of response? *JIMD Rep* 2013; 5: 59-70.
129. Xiao J, Westbroek W, Motabar O et al. Discovery of a novel noniminosugar acid alpha glucosidase chaperone series. *J Med Chem* 2012; 55: 7546-7559.
130. Feldhammer M, Durand S, Pshezhetsky AV. Protein misfolding as an underlying molecular defect in mucopolysaccharidosis III type C. *PLoS One* 2009; 4: e7434.