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## **New and potential strategies for the treatment of PMM2-CDG**

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## ***Abstract***

Mutations in the *PMM2* gene cause phosphomannomutase 2 deficiency (PMM2; MIM# 212065), which manifests as a congenital disorder of glycosylation (PMM2-CDG). Mutant PMM2 leads to the reduced conversion of Man-6-P to Man-1-P, which results in low concentrations of guanosine 5'-diphospho-D-mannose, a nucleotide-activated sugar essential for the construction of protein oligosaccharide chains. To date the only therapeutic options are preventive and symptomatic. This review covers the latest advances in the search for a treatment for PMM2-CDG. Treatments based on increasing Man-1-P levels have been proposed, along with the administration of different mannose derivatives, employing enzyme inhibitors or repurposed drugs to increase the synthesis of GDP-Man. A single repurposed drug that might alleviate a severe neurological symptom associated with the disorder is now in clinical use. Proof of concept also exists regarding the use of pharmacological chaperones and/or proteostatic regulators to increase the concentration of hypomorphic PMM2 mutant proteins.

***List of abbreviations:***

**AONs:** antisense oligonucleotides

**CDG:** congenital disorder of glycosylation;

**ER:** endoplasmic reticulum

**GDP-Man:** guanosine 5'-diphospho-D-mannose

**HSR:** heat shock response

**LLO:** lipid-linked oligosaccharide

**Man:** mannose

**Man 1P:** mannose 1 phosphate

**Man-6-P:** mannose 6 phosphate

**MPI:** phosphomannose isomerase

**PC:** pharmacological chaperones

**PLGA-NPs:** GDP-Man-loaded poly (D,L-lactide-co-glycolide) nanoparticles

**PMM2:** phosphomannomutase 2

**PR:** proteostatic regulators

**QCS:** quality control systems

## ***Introduction***

Mutations in the *PMM2* gene (MIM# 601785) cause phosphomannomutase 2 deficiency (PMM2; MIM# 212065), manifesting as a congenital disorder of glycosylation (CDG).

PMM2 is a homodimeric enzyme that catalyzes the conversion of mannose 6 phosphate (Man-6-P) to mannose-1-phosphate (Man-1-P) in the cytosol, generating a key substrate for N-glycan biosynthesis [1, 2]; the activator of this enzyme is glucose 1, 6 biphosphate. This is the first committed step in the synthesis of guanosine 5'-diphospho-D-mannose (GDP-man, a nucleotide-activated sugar essential for the construction of protein oligosaccharide chains) and dolichol-P-mannose, both of which are mannose donors. The synthesis of N-glycans, O-mannose-linked glycans, glycoposphatidylinositol anchors, and C-mannosylated proteins, all require these donors [3].

Cells transport exogenous mannose via facilitated diffusion hexose transporters belonging to the SLC2A group (GLUT), which are present (mainly) in the plasma membrane. Within the cell, mannose is phosphorylated by hexokinase (HK) to Man-6-P, which serves as a substrate for three competing enzymes: phosphomannose isomerase (PMI or MPI), KDN-9-phosphate synthetase (KPS) and PMM2. The fate of Man-6-P depends on the ratio of MPI to PMM2. Within cells, a higher ratio leads to the catabolism of Man-6-P while a lower ratio favors the glycosylation pathway. Once generated from Man-6-P, Man-1-P is incorporated into several glycosylation intermediates including GDP-man, GDP-fucose and dolichol phosphate mannose which go on to be N-glycosylated, O-glycosylated, C-mannosylated, or to be used in the synthesis of glycosylphosphatidylinositol anchors (Figure 1). Both exogenous and glycogen-derived

glucose can contribute to this pathway [4, 5]. Mutated PMM2 leads to the reduced conversion of Man-6-P to Man-1-P, which results in low concentrations of GDP-Man, a nucleotide-activated sugar essential for the construction of protein oligosaccharide chains [4, 5].

PMM2-CDG is the most common CDG with more than 1000 cases recorded worldwide [6]. A constellation of clinical symptoms has been described in patients with this problem. Mutations affecting PMM2 can give rise to phenotypes ranging from mild to severe with neonatal death [7]. In nearly all patients the nervous system is affected [8], with symptoms ranging from an inability to walk, to the lack of speech, poor comprehension, autistic features, and mild intellectual disability [9]. PMM2-CDG is also associated with a failure to thrive, gastrointestinal symptoms, hypotonia, developmental delay, cerebellar atrophy, epilepsy, strabismus and other movement disorders, liver disease and coagulopathy, pericardial effusion, endocrinological manifestations such as hypothyroidism and hypogonadotropic hypogonadism, osteopenia and lipodystrophy [8, 9]. Severe forms are often fatal during the first years of life; indeed, the global mortality rate during these early years can reach 20%. There is currently no cure for PMM2-CDG; only preventive and symptomatic treatments are available [10].

More than one hundred pathogenic genetic variants have been reported associated with PMM2-CDG worldwide, of which 80% are missense mutations according to the Human Gene Mutation Database (HGMD professional® 2020.1). The most common variant in all populations is p.Arg141His, a mutation always associated with other hypomorphic missense variants [11]. The second most common variant in some

populations is p.Phe119Leu, a missense mutation located in the dimerization region of the protein. Most patients are compound heterozygous and the most common genotype in most populations is p.Arg141His/p.Phe119Leu [6]. Some patients have up to 40% normal enzymatic activity, but heterozygous individuals retain at least 50% and are asymptomatic [4].

Research into treatments for PMM2-CDG has involved the use of different cellular and animal models. Indeed, much effort has been invested in developing zebra fish, fruit fly and mouse models for testing potential therapeutic molecules. A zebra fish morpholino knockdown model of PMM2-CDG showed what appeared to be disease-relevant phenotype [12]. Also, fly models of PMM2-CDG have been generated, with features that apparently resemble disease-relevant phenotypes, but they all suffer from early larval lethality. Recently, the first yeast models of PMM2-CDG have been reported [13], and in the last year the generation of the first worm model of PMM2-CDG was used to identify drug repurposing candidates able to boost PMM2 enzyme function [14]. A mouse model would, however, be much more desirable. Such a model might recreate all the features of the disease, from internal organ involvement to behavioral and neurological disturbances, but given that N-linked protein glycosylation occurs in all cell types throughout life, it would not be easy to mimic patient pathophysiology [15-17]. Two mouse models have been proposed, although they suffer from certain limitations. The first, a *Pmm2* hypomorphic mouse model with the mutations R137H and F118L, suffered from complete embryonic lethality [18] and is therefore severely limited in its usefulness. A compound heterozygous mouse with the p.R137H and p.F115L mutations, corresponding to the most prevalent alleles found in patients with PMM2-CDG, has also been generated, but many of these *Pmm2*R137H/F115L mice die prenatally and those

that survive show significantly stunted growth. Despite these drawbacks, this is the first clinically relevant mouse model of PMM2-CDG to have been developed [17].

The present review examines the strategies currently being explored for the treatment of PMM2-CDG.

### **Therapies designed to increase Man-1-P**

A number of strategies designed to circumvent the disruption of the glycosylation pathway through the rescue of the GDP-Man substrate, including nutritional sugar supplementation, the inhibition of enzymes that have Man-6-P as their substrate, and the upregulation of key enzymes involved in the production of Man-1-P have been proposed.

The rationale behind mannose supplementation therapy is that cells affected by PMM2-CDG have a reduced GDP-Man pool, and that exogenous mannose restores it, correcting the underglycosylation defect [19]. The nutritional administration of mannose has been tested, *in vitro* in patient-derived cell lines, and in patients [5]. However, despite increasing the blood levels of mannose without any side effects, no clinical or biochemical improvements were seen in PMM2 patients [20] [21] [22, 23]. It has been reported that while mannose supplementation in PMM2-CDG patients is unsuccessful, in MPI-deficient patients better results are obtained [22] [21, 24, 25]. The most likely explanation for the failure of mannose therapy in these patients is that the Man-6-P resulting from the increased mannose was not available because it was simply catabolized via MPI - the activity of this enzyme remains normal in PMM2-deficient patients.

Another explanation for the failure of mannose therapy in these patients was proposed after observations made in streptolysin O-permeabilized cells [26] [27]. The



addition of Man-6-P to these cells preferentially reduced the amount of the major lipid-linked oligosaccharide (LLO) species, Glc3Man9GlcNAc2-PP-Dol, resulting in the release of free glycan. Thus, increasing Man-6-P in either PMM2 or MPI-deficient cells could reduce the amount of available LLO and exacerbate the glycosylation defect. However, while providing mannose to either PMM2- or MPI-deficient cells might increase the GDP-mannose pool [19], showing that the metabolic flux could be increased, the Man-6-P pool would not be increased, nor would cellular glycosylation be reduced [4].

The direct correlation between phenotypic severity and enzyme dysfunction manifested by lower mannose peak concentrations suggests that the effectiveness of exogenous mannose supplementation is influenced by PMM2 residual activity [28-30]. It is important to note that an alternative transport system may be in operation. Certainly, metformin has been reported to help induce and increase mannose uptake by PMM2-deficient cells via the activation of a mannose-selective transport system, which corrects N-glycosylation in these cells [10, 31].

The effect of D-mannose supplementation in humans has been studied after its enteral administration in five patients and its parenteral use in one, but no clinical or biochemical improvement was observed [21, 22, 32]. In these studies, mannose was only administered for a few weeks, but recently a long-term trial of continuous D-mannose infusion was performed (continuous intravenous mannose over the first 5 months of life at a dose of 0.8 g/kg/day) - to investigate the clinical effect on PMM2-CDG [23]. However, no biochemical nor clinical effect was seen either. The authors of this study suggested that the period of treatment might have been too short, that larger doses of mannose might be needed, and that patient genotype may have interfered with the clinical and biochemical results.

The use of membrane-permeant derivatives of Man-1-P [33] and hydrophobic Man-1-P has also been explored, but given their poor pharmacokinetic properties, the synthesis of other, more stable and less toxic compounds is needed [34]. Strategies designed, to facilitate the uptake and incorporation of mannose, include the synthesis of membrane-permeable or hydrophobic mannose-1-phosphate-based prodrugs [34, 35]. The latter compounds were shown to correct glycosylation *in vitro* and may provide new therapeutic options.

Since mutated PMM2 leads to the reduced conversion of Man-6-P to Man-1-P, which results in low concentration levels of GDP-Man, GDP-Man-loaded poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) were proposed to treat PMM2-CDG, thus bypassing the glycosylation pathway reaction catalyzed by PMM2. The degree of hypoglycosylation achieved was studied in fibroblast cultures [36], measuring the activity of lysosomal enzymes, the function of which mainly depends on the oligosaccharide chains linked to asparagine amino groups. Assessment of the lysosomal enzymes allowed the determination of the dose, the incubation time, and the time required for the successful delivery of GDP-Man. Since PMM2-CDG provokes neurological disorders, PLGA nanoparticles would be particularly useful since they are able to cross the brain blood barrier when coated with a short glycopeptide that links to the endothelial opioid receptor [37, 38]. After 48 h, the specific activities of  $\alpha$ -mannosidase and  $\beta$ -galactosidase were estimated at 69% and 92% of that seen in control cells. The residual activity of  $\beta$ -glucuronidase increased from 6.5% to 32.5% and was significantly higher than that noted in untreated fibroblasts. These results show that treatment caused the reappearance of several glycosylated proteins, and that further preclinical evaluation is warranted [36].

Another therapeutic strategy proposed for PMM2-CDG is the inhibition of MPI. This relies on the fact that the majority of Man-6-P is catabolized by MPI, reducing the amount of this precursor available for glycosylation [39]. Based on the premise that the inhibition of MPI should provide more Man-6-P for glycosylation, the use of a potent MPI inhibitor has been proposed, i.e. MLS0315771 from the benzoisothiazolone series that diverts Man-6-P toward glycosylation in patient cell lines. Certainly, it increases the metabolic flux of mannose towards glycosylation in zebra fish embryos. However, even though an inhibitory effect on MPI was observed, some toxic effects for concentrations above 2  $\mu$ M were seen. Further compound optimization will be required to maintain MPI inhibition while reducing toxicity [29].

The challenge regarding sugar supplementation is delivery into specific tissues. Different methods have been developed, however, to facilitate the uptake and incorporation of mannose into cells, such as the synthesis of membrane permeable hydrophobic Man-1-P-based prodrugs [34, 35]. A company is also developing a Man-1-P formulation using liposomes as the intravenous delivery system, but these will probably not cross the blood-brain barrier [23] [10].

A drug repurposing screen has been described that uses a novel worm model of PMM2-CDG, followed by PMM2 enzyme functional studies in PMM2-CDG patient fibroblasts. Drug repurposing is a viable strategy for developing orphan drugs since it is associated with fewer risks, lower costs, and shorter timelines than other methods. Repurposed medicines have the added value of being able to go straight to clinical trials since safety is guaranteed (previously confirmed during investigations for their original indication) [40].

Lao *et al* [13] undertook a pilot drug repurposing study using a yeast model of PMM2-CDG that allows the genotype-phenotype relationships to be examined in a growth-based assay. The growth deficiency of the mutant alleles correlates with the enzymatic defect. The exercise allowed them to select three hits that restored the growth defect in the mutant strains. One of these three compounds is the  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA), a potent aldose reductase inhibitor (ARI) [13]

In addition, twenty drugs have currently been selected as repurposing candidates (all identified using worm models of PMM2-CDG), 12 of which are plant-based polyphenols. Work on their structure–activity relationships revealed the antidiabetic aldose reductase inhibitor epalrestat to be a first-in-class PMM2 enzyme activator [41]. Epalrestat increased PMM2 activity in four PMM2-CDG patient fibroblast lines with the genotypes p.R141H/p.F119L, p.R141H/p.E139K, p.R141H/p.N216I and p.R141H/p.F183S. PMM2 enzyme activity gains ranged from 30% to 400% over baseline, depending on the genotype. The pharmacological inhibition of aldose reductase by epalrestat may shunt glucose from the polyol pathway to glucose-1,6-bisphosphate, an endogenous stabilizer and coactivator of PMM2 homodimerization. The efficacy of epalrestat suggested that it could be given to patients with PMM2-CDG who are compound heterozygous for R141H plus any pathogenic variant. Epalrestat is the first small molecule activator of PMM2 with the potential to treat peripheral neuropathy and correct the underlying enzyme deficiency in PMM2-CDG patients [14]. The current drawback is that this compound has only been approved to treat diabetes mellitus in Asia; it has not been approved by the US FDA or European EMA.

**Therapies designed to increase the concentration of hypomorphic PMM2 protein**

The functional characterization of disease-causing mutations described in patients with PMM2-CDG led to the idea of using small molecules such as pharmacological chaperones (PC) to rescue PMM2 loss-of-function mutations. The unstable nature of certain PMM2 missense pathogenic variants causes protein instability, although some residual activity may be retained [11], showing PMM2-CDG to be a conformational disease [42] [43, 44]. Protein misfolding induced by missense mutations has been identified as the cause of many diseases [45]. Loss of protein function results from early degradation, mislocalization, structural alteration or aggregation leading to pathological dysfunctions [46]. When misfolded proteins cannot be properly refolded, the ubiquitin-proteasome system, autophagy and ER-associated degradation begin to degrade them [44]. Knowledge of the proteostasis network and protein quality control system (QCS) is, however, paving the way for new treatments for protein misfolding diseases. Influencing the proteostasis network, or directly stabilizing target proteins using PCs or proteostasis regulators (PRs - small compounds that increase the capacity of the proteostasis network by, for example, increasing the expression of chaperones and thus activating certain protective pathways that increase folding capacity [47]) offers the possibility of treating several severe illnesses [44].

The inherent instability described for most of the PMM2 mutants carried by patients with PMM2-CDG led our group to investigate compounds that might increase protein folding capacity in the cytoplasm (where PMM2 is located). PCs are interesting candidates for this [48]. These small molecules bind specifically to proteins to stabilize them and prevent their degradation and/or aggregation by promoting their correct folding. They are a promising therapeutic option for treating PMM2-CDG [43, 49-51]. PCs are able to cross the blood–brain barrier and may therefore help alleviate the

central nervous system problems associated with PMM2-CDG, as well as help treat a number of lysosomal storage disorders such as Gaucher and Fabry disease [44]. It has been suggested that this drug option could be successful in treating the problems of loss-of-function mutations, either through allosteric interactions or by inhibiting binding to the active site of the target protein [46]. Interestingly, increasing the amount of mutant protein by changing the native promoter for a stronger one has been reported to improve the growth of all yeast mutants studied, except for the R141H equivalent variant. This supports the idea that therapies targeted towards increasing the PMM2 concentration may be beneficial to patients [13].

Two methods of screening for PCs are available; one requires the screening of large libraries of molecules that can identify a lead compound for further refining, while the other involves modifying the structure of known ligands of an enzyme to improve their potency, safety and stability. Both strategies are valid and have demonstrated their efficacy by bringing drugs to the market [10]. Our group has undertaken high-throughput screening (differential scanning fluorimetry) to search for compounds that can increase the stability of PMM2 human recombinant protein. A commercial library of 10,000 small molecules was investigated, and eight compounds were identified that increased the thermal stability of PMM2. Of these, only one, 1-(3-chlorophenyl)-3-3-bis(pyridine-2-yl)urea (compound VIII), showed all the pharmacochemical characteristics necessary to be considered a viable candidate. This compound is a good starting point for producing optimized derivatives for transfer to the next step of drug development. These results provided the first proof-of-concept of a possible treatment for PMM2-CDG [43].

Structure-based virtual screening has recently led to the identification of new lead compounds. Indeed, while -(3-chlorophenyl)-3-3-bis(pyridine-2-yl)urea (compound VIII) shows high binding affinity for p.D65Y mutations, two new hits have been identified that show greater affinity for other mutant proteins. Different mutations may therefore be better targeted by different PCs [52].

Using the second of the mentioned strategies for screening pharmacological compounds,  $\alpha$ -glucose-1,6-bisphosphate, an analog of the natural ligand of PMM2, was proposed as a potential PC for addressing unstable PMM2 mutants.  $\alpha$ -glucose-1,6-bisphosphate might be thought the ideal chaperone for rescuing unstable pathological variants, but it is hydrolyzed by PMM1, and for this reason an analogous biphosphate sugar, the anomer  $\beta$ -glucose-1,6-biphosphate, was investigated. It acts as a typical chaperone since it is a mildly non-covalent inhibitor of PMM2, and its effect *in vitro* was demonstrated in selected destabilizing mutants. Currently, efforts are being made to enhance its bioavailability through the use of hydrophobic derivatives or associating it with liposomes [51].

Proteostasis requires that a delicate balance be maintained between protein synthesis, folding, trafficking and degradation. The cell is thus equipped with protein QCS in which chaperone molecules, the ubiquitin proteasome pathway, and autophagy play important roles [53]. In recent years, PRs have been shown to modulate protein function in different conformational diseases by stabilizing defective, misfolded proteins [46]. Enhancing intracellular proteostasis capacity by PRs could be used against many different disorders with the same underlying pathogenic mechanism. Different compounds for treating conformational diseases and neurodegenerative disorders are currently in the preclinical and clinical stages of investigation [44].

Celastrol is a well-known PR that modulates the proteostasis network through the activation of the heat shock response (HSR), and it has returned positive results in a cellular model of PMM2-CDG. A significant increase in PMM2 protein levels and activity was detected after treatment in four cell lines overexpressing either the oligomerization mutation p.D65Y, p.R162W or p.T237M, or the dimerization mutation p.F119L. An increase was seen in several molecular chaperones of the heat shock protein family, both at the transcriptional and proteome level. The use of specific inhibitors of Hsp70 and Hsp90 suggested that the latter plays a key role in the celastrol-induced stabilization of PMM2 mutants. This study provides proof-of concept for the use of stabilizing molecules in the treatment of PMM2-CDG [54].

However, PCs that better increase the amount of PMM2 are needed. In the new era of drug discovery, disruptive computational technologies are helping select compounds for evaluation in cellular models [43, 55]. Recent progress in artificial intelligence and machine learning methods [56] are also having a tremendous impact on the search for novel bioactive small molecules, cutting discovery times to a fraction of what they were just a few years ago [57, 58]. One of the most important works recently published in this field describes the design - in just 21 days - of potent inhibitors of discoidin domain receptor 1 (DDR1), a kinase target implicated in fibrosis [57]. The full characterization of the three-dimensional structure of the PMM2 protein will provide further clues that might accelerate drug discovery [59].

A major challenge is to know how many patients with PMM2-CDG might be amenable to drug-based rescue. It should be remembered that all patients show some residual activity. Since a high number of hypomorphic variants likely affect PMM2 stability, many patients might benefit from PCs.



### **Therapies based on pathophysiology**

Acetazolamide, a drug successfully used in patients with gain-of-function Ca<sub>v</sub>2.1 channel activity, has been repurposed as a treatment for PMM2-CDG. It is a carbonic anhydrase inhibitor, that probably changes the intracellular pH, and thereby the transmembrane potential, which must affect the inactivation of calcium channels. Thus, acetazolamide may restore the excitability and resting activity of neurons [60].

The AZATAX phase II clinical trial ([www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu) Identification number: 2017-000810-44) was intended to establish whether acetazolamide could be repurposed to treat cerebellar impairment in PMM2-CDG. PMM2-CDG causes cerebellar syndrome and stroke-like episodes that are also described in patients with gain-of-function mutations in the Ca<sub>v</sub>2.1 channel, for which acetazolamide therapy is used. *In vitro* impairment of the N-glycosylation of Ca<sub>v</sub>2.1 promotes a similar gain-of-function effect and may reflect the situation in PMM2-CDG cerebellar syndrome [61]. The safety of acetazolamide and its effectiveness against PMM2-CDG cerebellar syndrome [62] was determined using the International Cerebellar Ataxia Rating score [63], a dysarthria scale (the PATA rate) [64], the Nijmegen Pediatric CDG score [65], and several psychometric assessments and quality of life scales [62]. The trial then focused on the motor and cognitive aspects of cerebellar syndrome since neurological dysfunction is the main cause of long-term disability, daily life limitations, and reduced autonomy in PMM2-CDG. An unexpected normalization in coagulation parameters was also seen [62].

The AZATAX trial showed acetazolamide to be well tolerated in most patients, and to be effective against the motor and cognitive features of the cerebellar syndrome [62]. It has no known contraindication, nor is it known to interact with other medications.

### **Antisense therapy**

RNA therapeutics has increased the “druggable” space traditionally focused on small molecule- or protein-based therapies. RNA therapies are easy to design, cost-effective, and have already been proven a viable path for personalizing the treatment of rare diseases {Crooke, 2018 #87;Yin, 2019 #93;Kim, 2019 #96;Kim, 2019 #2548}. RNA-based therapeutics involves the use of antisense oligonucleotides (AONs) [66, 67], some 15-30 nucleotides in length, which avoid the challenges of viral vector-mediated delivery required by gene therapy and genome editing. Eight AONs are already approved for clinical use, although challenges to broad translation into the clinic persist, including poor pharmacological properties and difficulties in delivery to specific target organs and tissues. Chemically synthesized AONs bind to only their target RNA following Watson-Crick pairing rules. Following binding, and depending on chemical and positional requirements, AONs modulate RNA function by steric blocking mechanisms (blocking translation or modulating splicing), or by promoting RNA cleavage and degradation (RNAse H1 or Ago2 recruitment) [67].

Given that the disruption of correct splicing by point mutations represents about 15% of all the mutations described in the Human Gene Mutation Database, antisense therapy is an attractive option. The disruption of conserved intron-exon junction sequences or other intronic regions can lead to the activation of cryptic splice-sites and the inclusion of intronic sequences.

Several mutations affecting normal *PMM2* mRNA splicing have been identified [42] [68, 69]. Antisense therapy using morpholino oligonucleotides has been tested against these problems in patient-derived fibroblasts. Using AMOs targeting the donor and acceptor cryptic splice sites of a pseudoexon activated by c.640-15479C>T

mutation`, patient-derived fibroblasts began to show a normal splicing profile 24 h after transfection in a sequence- and dose-dependent manner. Protein levels and enzymatic activity were increased by 30% and 40% respectively [42].

Several AONS have already been approved for clinical use, but their poor pharmacological properties and the difficulty in targeting specific organs, including the brain, currently reduce their potential [70, 71].

### **Key challenges**

Despite substantial efforts to develop therapies for PMM2-CDG, only one drug has reached the clinic (Figure 1 and Table 1). Indeed, the development of therapeutic strategies for PMM2-CDG faces several key challenges. These include: (i) the presence of such a wide mutational spectrum that nearly every patient has his or her own double heterozygous combination [6]; (ii) the lack of animal models mimicking human clinical conditions upon which to perform preclinical experiments and evaluate improvements, (iii) and the absence of correlation between serum biomarkers (sialotransferrin profile, coagulation factors, etc.) and clinical severity.

For a therapy to reach the clinic, good mutation-specific preclinical PMM2 models must be available. Those currently at hand all have limitations, including their failure to replicate certain symptoms. The use of stem cells from PMM2-CDG patients showing a gradual reduction in N-glycosylation would certainly improve the cellular and molecular study of PMM2 deficiency [55]. In fact, the generation of patient-derived iPSCs and 3D organoids is now opening up the investigation of the mechanisms influencing embryonic development and organ-specific disease, as well as providing new material for testing therapies [72] [73].

The success of small molecules in treating PMM2-CDG might be improved by better disease modeling. To date, studies on single aspects of the disease have provided useful insights into its origin. However, the human organism involves a complex network of pathways, and even though PMM2-CDG is a monogenic defect, several pathways are affected. The useful aspect in this is that any of these could be a potential target for drug discovery. Studies based on systems biology, analyzing the problem from multiple perspectives, could therefore be more powerful in discerning the downstream consequences of mutations and any potential treatments [74]. The development of novel algorithms capable of integrating multi-omic data (i.e., metabolomic, transcriptomic, phenomic and proteomic data) should facilitate the identification of functional pathways for targeting. Systems biology approaches for the study of PMM2-CDG might also allow the proposal of novel mechanistic hypotheses [75].

Systems biology could also help identify novel biomarkers for diagnosing PMM2-CDG, as well as for monitoring disease progression and response to treatment [10, 76]. Dynamic computational models may also be valuable in optimizing treatment for each patient by taking into account specific cellular signatures, predicting drug efficacy, resistance, and adverse effects, as well as in identifying the most effective dose or synergic combination of drugs while minimizing these adverse events [77].

Systems biology can also be used in drug repurposing. Computational approximations to drug repurposing involve virtual screening using *in silico* experimental models and machine learning to find new relationships between candidate compounds and clinical observations.

In conclusion, the advances made in recent years have opened up new and promising therapeutic avenues for treating PMM2-CDG. Hopefully, in the next few years

the possibilities discussed above will bring new compounds to the clinic that might more effectively treat patients with this orphan disease.

**Figure 1: Pharmacological options described for PMM2-CDG.** Man = Mannose; Man-6-P = Mannose 6 phosphate; Man-1-P = Mannose 1 phosphate; Glu = Glucose; Glu-1,6-BP = Glucose 1,6 bisphosphate;  $\beta$ -Glu-1,6-BP = beta Glucose 1,6 biphosphate; GDP-Man = GDP-mannose; KDN-9-P = 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid; HK = Hexokinase; KPS = KDN-9-phosphate Synthetase; MPI = Mannose-6-phosphate isomerase; AR = Aldose Reductase; CaV2.1 = Voltage-dependent P/Q-type calcium channel subunit alpha-1A; PC = pharmacological chaperone; CHCA  $\alpha$ -cyano-4-hydroxycinnamic acid. Pharmacologic/therapeutic options are represented by pill-icons.

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**Table 1.** Therapeutic strategies for treating PMM2-CDG

Therapeutic strategy	Aim	Rationale	Effect	Clinical relevance	Ref.
Mannose supplementation	Increasing the GDP-Man pool	Exogenous mannose restores the reduced GDP-Man pool correcting underglycosylation	Mannose therapy led to no extra Man-6-P becoming available to patients	Neither clinical nor biochemical improvement were seen in patients	[19] [22] [33] [23]
GDP-Man-loaded poly (D,L-lactide-co-glycolide) nanoparticles (PLGA NPs)	Delivery of GDP-Mannose	Reduced conversion of Man-6-P to Man-1-P results in low levels of GDP-Man essential for glycosylation	Reappearance of several glycosylated proteins	Desirable efficacy <i>in vitro</i> . Further preclinical evaluation warranted.	[36]
Inhibition of MPI	Inhibiting MPI, since the majority of Man-6-P is catabolized by MPI, reducing the amounts of Man-6-P available for glycosylation.	Phosphomannose isomerase (MPI) inhibition	Increases metabolic flux of mannose towards glycosylation	Toxic effects at low concentrations	[39] [29]
Aldose reductase inhibitor: epalrestat	PMM2 enzyme activator	The inhibition of aldose reductase prevents the transformation of glucose into sorbitol, increasing the potential to form glucose 1,6-bisphosphate, an endogenous stabilizer and coactivator of PMM2	Potential to treat peripheral neuropathy and correct the underlying PMM2 deficiency in patients	Epalrestat is only approved to treat diabetes in Asia; it is not approved by the FDA nor EMA	[14]
Pharmacological chaperones	PMM2 missense pathogenic variants causing protein instability while retaining some residual activity	Stabilizing target misfolded proteins by preventing early degradation, mislocalization, structural alteration or aggregation	Increase the concentration of the hypomorphic PMM2 protein	Promising preclinical studies directed towards improving the bioavailability and efficacy of the reported PCs (compound VIII and chemical analogs, $\alpha$ -glucose-1,6-biphosphate)	[51] [52] [43]
Proteostasis regulators (celastrol)	Increase in several molecular chaperones of the heat shock protein family	Enhancing intracellular proteostasis capacity of the proteostasis network	Increase the concentration of the hypomorphic PMM2 protein	Positive results in a cellular model of PMM2-CDG	[54]
Acetazolamide	Acetazolamide, a carbonic anhydrase inhibitor, has an inactivating effect on calcium Cav2.1 channel.	Impairment in N-glycosylation of Cav2.1 promotes channel gain-of-function and cerebellar dysfunction	Acetazolamide restores motor and cognitive aspects of the cerebellar syndrome	A potential synergistic treatment	[62]
Antisense therapy	Morpholino oligonucleotides	The use of RNA-based therapeutics would	Patient-derived fibroblasts treated with	Promising <i>in vitro</i> results	[42]

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directed toward  
pseudoexons or  
activation of cryptic  
splice sites

rescue mutations  
affecting normal  
PMM2 splicing

morpholino  
oligonucleotides  
rendered a normal  
splicing profile. Protein  
levels and enzyme  
activity were partially  
restored

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