Quantitative Systems Pharmacology-Based Digital Twins Approach Supplements Clinical Trial Data for Enzyme Replacement Therapies in Pompe Disease

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Pompe disease is a rare, progressive neuromuscular disease caused by deficient lysosomal glycogen degradation, and includes both late-onset (LOPD) and severe infantile-onset (IOPD) phenotypes. Due to very small patient numbers in IOPD and the high phenotypic heterogeneity observed in this population, a quantitative systems pharmacology (QSP)-based "digital twin" approach was developed to perform an *in silico* comparison of the efficacy of avalglucosidase alfa vs. the standard of care, in a virtual population of IOPD patients. A QSP model was developed that represents key elements of Pompe disease pathophysiology, including tissue glycogen accumulation and the elevation of the biomarker urine Hex4 in both LOPD and IOPD patients. In this approach, the QSP model was used to generate digital twins of each IOPD patient enrolled in the avalglucosidase alfa clinical program, considering their respective disease burden, demographics, and treatment history. This virtual cohort supplemented clinical observations by simulating and comparing tissue glycogen and urine Hex4 following avalglucosidase alfa treatment vs. the standard of care. The digital twin analysis supports the interpretation that the enhanced reduction in urine Hex4 observed following avalglucosidase alfa treatment is attributable to greater tissue glycogen clearance. Overall, this study provides mechanism-based insight into avalglucosidase alfa efficacy across the phenotypic spectrum of Pompe disease and demonstrates the value of applying a QSP-based digital twin analysis to support rare disease drug development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Pompe disease has a broad spectrum of clinical phenotypes, with varying ages at symptom onset, rates of clinical progression, and overall clinical severity, which creates challenges in interpreting efficacy data.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We developed the first multiscale quantitative systems pharmacology (QSP) model that mechanistically connects the deficiency in lysosomal glycogen degradation to glycogen accumulation and biomarker profiles in plasma and urine. A digital twin-based analysis was performed to compare the biomarker efficacy of standard of care and next-generation enzyme replacement therapies (ERTs).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The QSP model captures both late-onset (LOPD) and infantile-onset (IOPD) populations, supporting the continuity of the phenotypic spectrum. The model is also able to recapitulate the range of variability observed in untreated patients, and their response to ERT.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This QSP analysis provides mechanistic insight into ERT response across the phenotypic spectrum of Pompe disease. This study highlights the value of applying a digital twin-based approach to support rare disease drug development.

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Pompe disease (also known as acid maltase deficiency or glycogen storage disease type II) is a rare, autosomal recessive genetic disease caused by the deficiency of lysosomal acid α-glucosidase (GAA), an enzyme that degrades glycogen. The resulting accumulation of glycogen in body tissues, especially cardiac and skeletal muscles, disrupts the architecture and function of affected cells, leading to a variety of debilitating multi-systemic symptoms, clinical decline, and in many cases, premature death.

Pompe disease has a broad spectrum of clinical phenotypes, with varying ages at symptom onset, rates of clinical progression, degrees of organ involvement, and overall clinical severity.[1–4] In the more severe and rapidly progressive infantile-onset Pompe disease (IOPD) form, patients experience symptom onset at < 12 months of age with cardiomyopathy. If untreated, IOPD is fatal in infancy. The more slowly progressive late-onset Pompe disease (LOPD) is defined by symptom onset at < 12 months of age without cardiomyopathy, or onset at ≥ 12 months of age. In some cases, patients do not experience symptom onset until adulthood.

The enzyme replacement therapy (ERT) approach for treating Pompe disease consists of recombinant enzyme supplementation of the deficient endogenous enzyme to break down accumulated glycogen. Alglucosidase alfa (commercialized as Myozyme® or Lumizyme®) is a recombinant human acid α-glucosidase (rhGAA) that contains low levels of mannose-6-phosphate (M6P) that mediates cellular uptake and lysosomal targeting via the cation independent M6P receptor (CIMPR). Avalglucosidase alfa (commercialized as Nexviazyme® or Nexviadyme®) is the next-generation rhGAA glycoengineered to have increased bis-M6P levels (~7 bis-M6P per GAA molecule) for optimal CIMPR binding and thereby improved targeted cellular uptake. Both alglucosidase alfa and avalglucosidase alfa are approved for the treatment of patients with LOPD or IOPD in the E.U. and other countries. In the United States, alglucosidase alfa is approved for patients with LOPD or IOPD, while avalglucosidase alfa is approved for patients with LOPD.

A quantitative systems pharmacology (QSP) model of Pompe disease was developed that mechanistically describes glycogen dynamics, integrating multiple scales of biology from the molecular-pathway-level processes driven by the GAA deficiency to the urine biomarker, hexose tetrasaccharide (Hex4), a metric of accumulated glycogen in tissue. This framework encompasses the mode of action of ERTs by accounting for the combined activity of supplemental and endogenous GAA. The model was further informed by clinical studies in both IOPD and LOPD patients, as well as from published natural history and real-world data[5] (more details provided in **Supplement**). The model was applied to perform an *in silico* comparison of alglucosidase alfa and avalglucosidase alfa efficacy in a virtual population of IOPD patients. Given the small patient number and the high heterogeneity of the disease, a QSP-based digital twin approach was implemented to generate "personalized" versions of the model (individual patient calibrations of the model) of each IOPD patient enrolled in the avalglucosidase alfa clinical development program. By leveraging the digital twins methodology, this analysis addresses disease heterogeneity and variable ERT response in patients with IOPD when comparing these treatments and can be used to supplement clinical trial datasets.

METHODS

The Pompe QSP model was developed by integrating a diverse set of data sources, including several clinical studies of avalglucosidase alfa and alglucosidase alfa. These data are described in detail in the Supplementary Methods (**Table S1**), including how they were used during model development.

The Pompe QSP model connects lysosomal GAA deficiency to glycogen and urine Hex4 dynamics in a semi-mechanistic manner to provide a multiscale representation of Pompe disease. Due to the continuity of LOPD and IOPD along a spectrum of phenotypic severity, the same model represents both populations, considering demographic differences and degree of enzyme deficiency. ERT is represented in the model as additive to endogenous residual GAA enzymatic activity, allowing the depiction of the mode of action of alglucosidase alfa and avalglucosidase alfa appropriately. Lysosomal ERT exposures for all regimens were derived from a physiologically-based pharmacokinetic (PBPK) model of both ERTs that considered clinical trial and preclinical data as well as a previously developed population pharmacokinetic (popPK) model.[6,7]

A modular approach was used to describe the intracellular synthesis and degradation of glycogen within appropriate organelles in representative cells of tissues of interest and the consequent production of the biomarker Hex4 (reported in plasma and urine matrices) from glycogen breakdown products. A schematic of the processes described in the model is shown in **Figure 1**.

The same molecular model was replicated to represent three different cell types: skeletal muscle fibers, cardiac muscle fibers, and hepatocytes. These three cell types were selected to represent organs key in the pathophysiology of Pompe disease. Skeletal muscle fibers are reported to be laden with glycogen and contribute to the muscle weakness and damage that characterizes Pompe disease. In the heart, accumulated glycogen in cardiac fibers gives rise to the hypertrophic cardiomyopathy observed in the most severely affected patients (IOPD phenotype).[8] Hepatocyte representations were also included in the model given their important role in glycogen homeostasis. To represent each tissue type appropriately, age- and sex-dependent volumes were referenced based on published data.[9–12]

Healthy glycogen metabolism is the result of a complex balance between biosynthesis and degradation reactions occurring in the cytoplasm and lysosome of the cell. Due to the complexity of these processes, it may not be feasible to represent all details in a model.[13] To maintain a parsimonious and fit-for-purpose representation, only reactions that were directly impacted by Pompe disease and/or the activity of the ERT (i.e., key elements of glycogen metabolism, including its breakdown by lysosomal GAA) were explicitly represented. Other reactions needed to maintain biological plausibility and mass order balance were lumped together and represented as degradation or production terms (e.g., glycolysis reactions that consume glucose-6-phosphate). Tissue-specific enzyme concentrations were included to represent glycogen dynamics in each tissue type appropriately, based on published data and public databases.[9–12]

The molecular scale of the model is initiated with glucose entry into the cell from blood via the tissue-specific glucose transporters, followed by the conversion of glucose into glucose-6-phosphate by hexokinase (HK).[13] Levels of glucose and glucose-6-phosphate within the intracellular space were constrained by published preclinical and clinical data.[14–16] In order to account for the tissue-specific differences in glycogen accumulation observed in Pompe disease, the synthesis of glycogen mediated by glycogen synthase (GYS1/GYS2) and the extralysosomal degradation catalyzed by tissue-specific glycogen phosphorylase (PYGL/PYGM/PYGB) are explicitly described. Other related enzymatic reactions, such as the activity of glycogen debranching enzyme, are lumped to represent a single compartment-specific degradative process.

In healthy individuals, glycogen synthesized in the cytoplasm is transported into the lysosome, where it is degraded to glucose by GAA. This is the key reaction that is deficient in Pompe disease and that is restored through supplementation with avalglucosidase alfa or alglucosidase alfa.

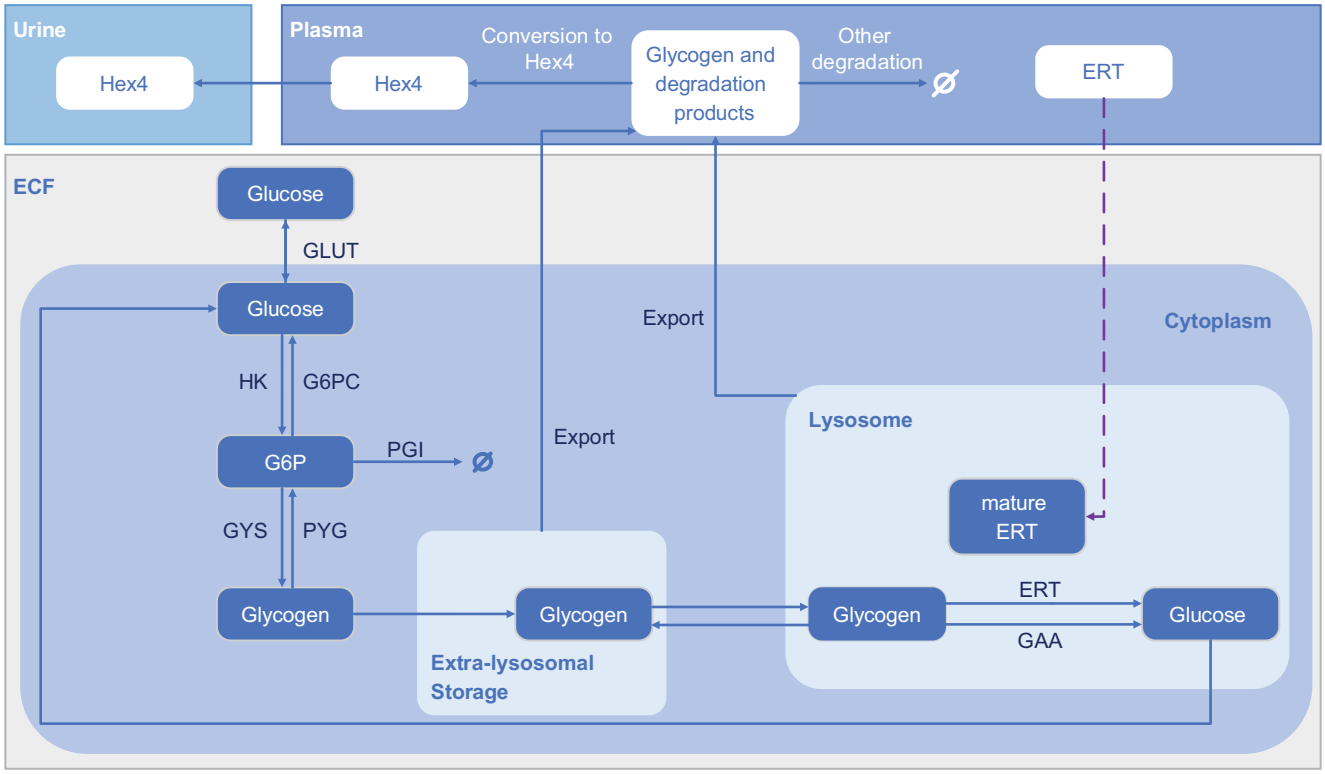

Figure 1 Structure of the QSP Model for Pompe disease (LOPD and IOPD) and the response to ERT (alglucosidase alfa or avalglucosidase alfa). The intracellular portion of the diagram describes a representative cell. The same structure is used to represent intracellular dynamics in skeletal muscle fibers, cardiac muscle fibers, and hepatocytes, using tissue-specific enzyme concentrations. Solid lines indicate reaction and transport processes, while the dashed line represents ERT transport into the lysosome described by the PBPK model[7]. Blue and white boxes are used to distinguish ECF and cytoplasmic species from those in plasma and urine. ERT, enzyme replacement therapy; GAA, acid α-glucosidase; G6PC, glucose-6-phosphate catalytic subunit; GLUT, glucose transporters; GYS, glycogen synthase; HK, hexokinase; IOPD, infantile-onset Pompe disease; LOPD, Late-onset Pompe disease; PGI, phosphoglucose isomerase; PYG, glycogen phosphorylase; QSP, quantitative systems pharmacology.

Due to the explicit representation of this GAA reaction deficiency, the model is able to describe variations in the degree of disease severity as variations in residual enzymatic activity (REA) of the endogenous GAA reaction. The REA is the key parameter calibrated to generate digital twins of clinical patients. In IOPD patients, a "responsiveness" parameter affecting ERT efficacy was also explored, which is described in more detail in the section on the development of IOPD digital twins. Additional details on model structure and parametrization (**Tables S2–S5**), including the ranges and sources of parameters and calibration procedures are provided in the Supplementary **Methods**.

RESULTS

Model appropriately represents key intermediates of healthy glycogen metabolism and the impact of moderate to severe GAA deficiency

The QSP model represents the production of glycogen from precursors glucose and glucose-6-phosphate (G6P), its cytoplasmic degradation, and its lysosomal degradation by GAA (**Figure 1**). The first step of model development was to recapitulate the dynamics of glycogen in healthy individuals by leveraging data in the public domain, as this allows the proper description of the relevant intracellular enzymatic reactions. As shown in **Figure 2a-c**, the model at steady state in a healthy virtual population adequately captures the levels of glycogen and key precursors in skeletal muscle reported in healthy individuals.[16–18] The healthy virtual population was generated through variability in body weight and height associated with sex and age, per the CDC growth chart[19]. Hex4 is a limit dextrin from the degradation of glycogen by non-specific amylases and is observed in both plasma and urine.[20,21] Hex4 is present at low concentrations in healthy individuals, and elevated urine Hex4 has been established as a biomarker for Pompe disease.[20,21] Through its description of exported glycogen degradation products, the QSP model's healthy virtual population describes the levels of Hex4 in urine in healthy individuals (**Figure 2d**).

Model recapitulates urine Hex4 profile in treatment-naive LOPD patients and treatment response to alglucosidase alfa and avalglucosidase alfa

Since Pompe disease is monogenic and its etiology clearly defined, it was assumed that representation of the Pompe disease state solely through the GAA deficiency using the healthy state model as a base was appropriate. This was accomplished by reducing the REA parameter, which describes the percent of healthy levels of GAA enzymatic activity that remains in the disease state due to the patient's mutation. It is reported that in LOPD patients REA can range from 2 to 40% of healthy

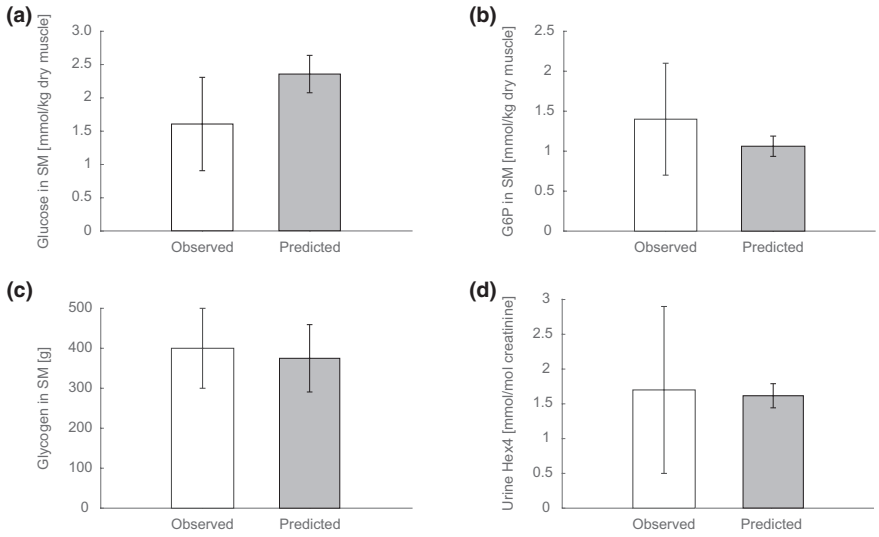

Figure 2 Simulation of glycogen metabolism in healthy individuals. Comparison of observed and predicted glycogen precursors (**a**) glucose and (**b**) glucose-6-phosphate in skeletal muscle of healthy individuals. (**c**) Predicted glycogen from the skeletal muscle in healthy individuals compared with observed data.[14–16] (**d**) Observed and predicted urine Hex4 in healthy individuals.[24,45] Mean and standard deviation of observed data and simulations are shown, and predictions are based on a healthy adult virtual population. G6P, glucose-6-phosphate; Hex4, hexose tetrasaccharide; SM, skeletal muscle.

levels, whereas in IOPD patients, the REA is < 2%.[22,23] As the REA of GAA was decreased into the LOPD range, simulated glycogen levels accumulated in skeletal muscle and other tissues as expected, and simulated urine Hex4 reached levels in accordance with reported literature and clinical trial observations for untreated LOPD patients (**Figure 3a**).[24,25]

The model was then calibrated to each individual LOPD patient enrolled in the COMET clinical study[25] (descriptions of all clinical studies included in model development and validation, are provided in **Table S1**) to generate digital twins, *in silico* representations of real patients which capture their demographics, disease history, and severity. A single digital twin was developed for each patient by optimizing the REA of GAA within the LOPD range based on the observed urine Hex4 at baseline, using the demographic characteristics of each individual to constrain intermediate calculations involving organ volumes and urine creatinine levels. This approach results in the expected relationship between REA and urine Hex4, with lower REA values giving rise to higher baseline urine Hex4 values (**Figure 3b**). Overall, simulations of the alglucosidase alfa and avalglucosidase alfa arms of the COMET trial showed good alignment with observed urine Hex4 trajectories during the 49-week double-blind treatment period (**Figure 3c**). The goodness-of-fit (GoF) plot comparing the corresponding predicted and observed data points from the alglucosidase alfa and avalglucosidase alfa arms of the COMET trial demonstrated that the model adequately captured the observed pre- and post-treatment levels of urine Hex4 in this LOPD patient cohort (**Figure 3d**).

The model was subsequently validated using three LOPD datasets not used during model development. First, it was applied to simulate the COMET extension study, in which adult patients receiving alglucosidase alfa during the double-blind treatment period of COMET were switched to avalglucosidase alfa.[26] The model captured the continued decline in urine Hex4 observed in these patients through week 121 (**Figure 4a**). Additionally, the model adequately represented urine Hex4 treatment response in hold-out data from the Ph3 study in alglucosidase alfa[27] and real-world data from the Pompe Registry,[5] as shown in **Figure 4b,c**. These comparisons with independent LOPD datasets support the robustness of the Pompe disease biology represented in the model. These validation results support the suitability of the disease processes described in the model and provide the level of confidence needed for the model to be applied to represent IOPD, where the patient population is much smaller.

Model recapitulates skeletal muscle glycogen and urine Hex4 profiles in pediatric IOPD patients and treatment response to alglucosidase alfa and avalglucosidase alfa

The QSP model was further extended to describe IOPD patients by simulating the lower REA range characterizing this phenotype (< 2%), as well as by scaling physiological parameters such as organ volumes to reflect the younger age range and body weight of these patients. The model adequately captures the clinically observed elevation of urine Hex4 in IOPD patients compared to age- and sex-matched healthy individuals (**Figure 5a**).[18,28] As in LOPD, further reduction of the REA within the IOPD range was associated with a further increase in urine Hex4 (**Figure 5b**).

The model was then applied to develop digital twins of the IOPD patients enrolled in Mini-COMET. Mini-COMET was a Ph2 study comparing alglucosidase alfa and avalglucosidase alfa in IOPD patients who were previously treated with alglucosidase alfa but demonstrated clinical decline or sub-optimal clinical response (**Table S1**).[29] The sub-optimal response in certain patients showed elevated urine Hex4 levels despite long-term alglucosidase alfa treatment.[11] Treatment history informed the generation of

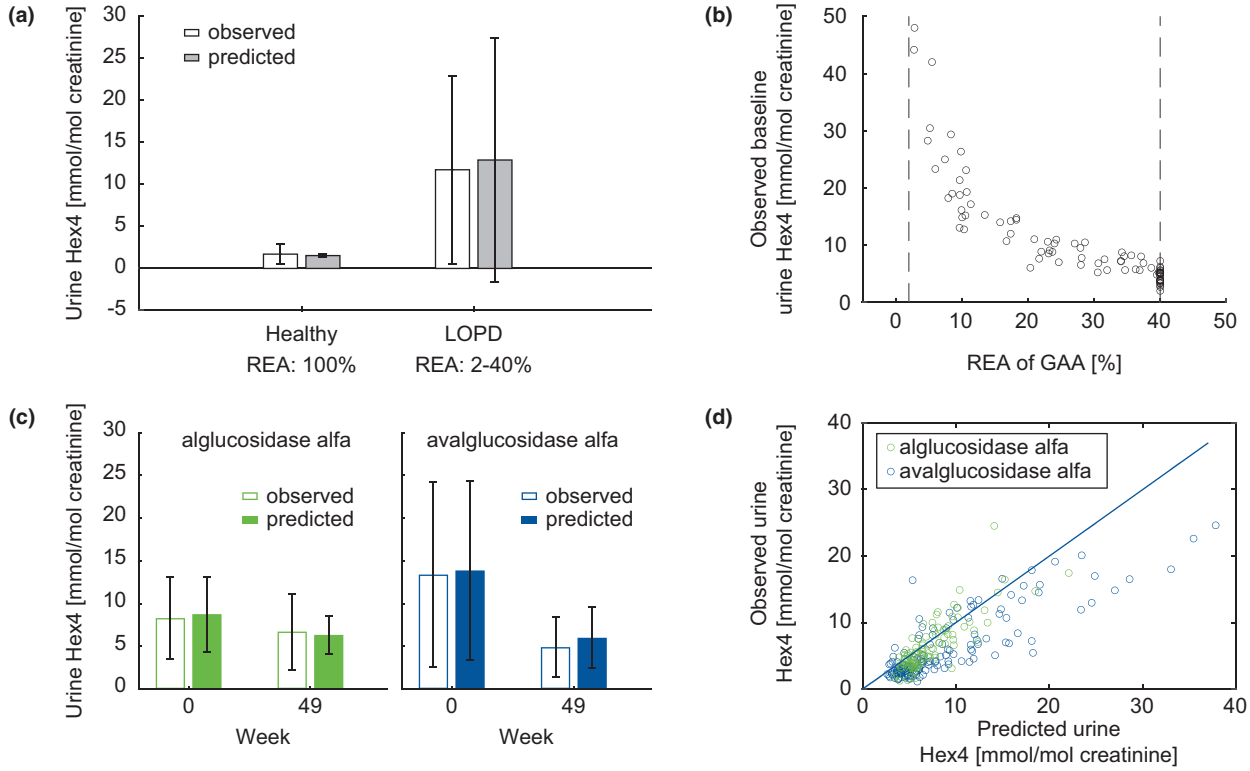

Figure 3 Development of LOPD digital twins. (**a**) Comparison of urine Hex4 (mean and standard deviation) predicted in LOPD adults and healthy adults with published observations[24,45] by varying the REA within indicated ranges. (**b**) Relationship between individually calibrated REA of GAA values and baseline urine Hex4 values for patients enrolled in the COMET[25] clinical study. (**c**) Comparison between simulations and observations of calibrated baseline and predicted week 49 urine Hex4 data from both arms of COMET (mean and standard deviation). (**d**) GoF of urine Hex4 at weeks 13, 25, and 49 for patients in both arms of COMET (RMSE = 3.8). GAA, acid α-glucosidase; Hex4, hexose tetrasaccharide; LOPD, Late-onset Pompe disease; REA, residual enzymatic activity; RMSE, Root Mean Square Error.

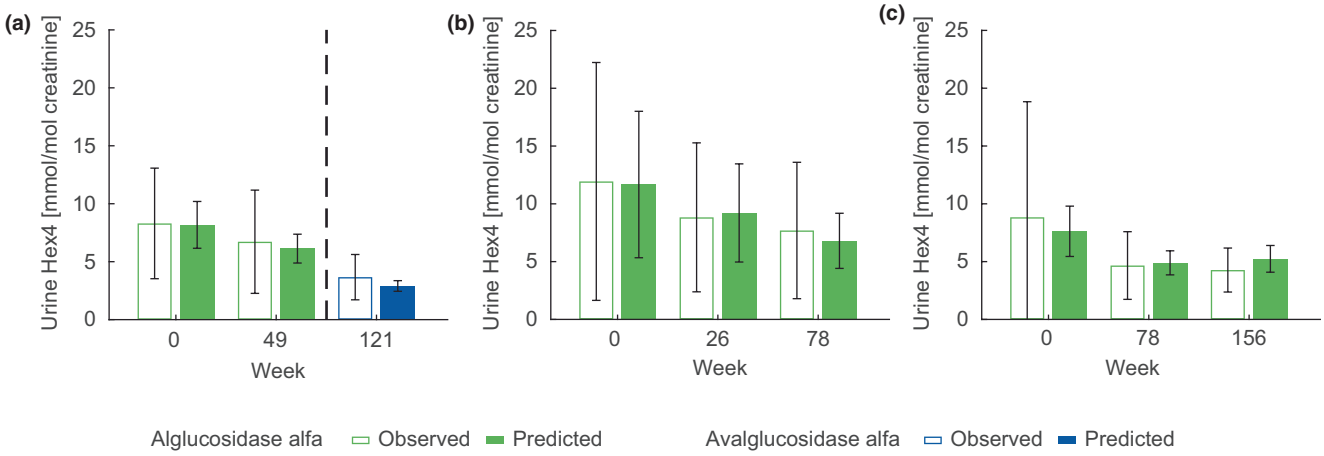

Figure 4 Validation of LOPD model using datasets not used in model development. (**a**) Validation of predicted (shaded) and observed (line) urine Hex4 time course in LOPD patients switched to avalglucosidase alfa after 49 weeks of treatment on alglucosidase alfa, using digital twins from the alglucosidase alfa arm of COMET. Model validation using a virtual population developed from the COMET digital twins simulated with 20 mg/kg EOW and compared against (**b**) hold-out data from the Phase 3 study[27] not used in model development and (c) independent data from all LOPD patients treated with alglucosidase alfa in the Pompe Registry.[5] Mean and standard deviation are shown in all subplots. The number of patients represented in the data overlays ranged from $N = 2$ to $N = 5$. EOW, every other week; LOPD, Late-onset Pompe disease.

digital twins to account for the heterogeneous response to alglucosidase alfa. It is hypothesized the sub-optimal response seen in these patients is due to insufficient clearance of skeletal muscle glycogen by alglucosidase alfa, leading to continuous glycogen accumulation and progressive muscle damage.[28,30,31] The reasons for insufficient glycogen clearance are unclear and may be attributable to disease-related factors such as progressive destruction of lysosomes and skeletal tissue that prevent effective uptake of ERT

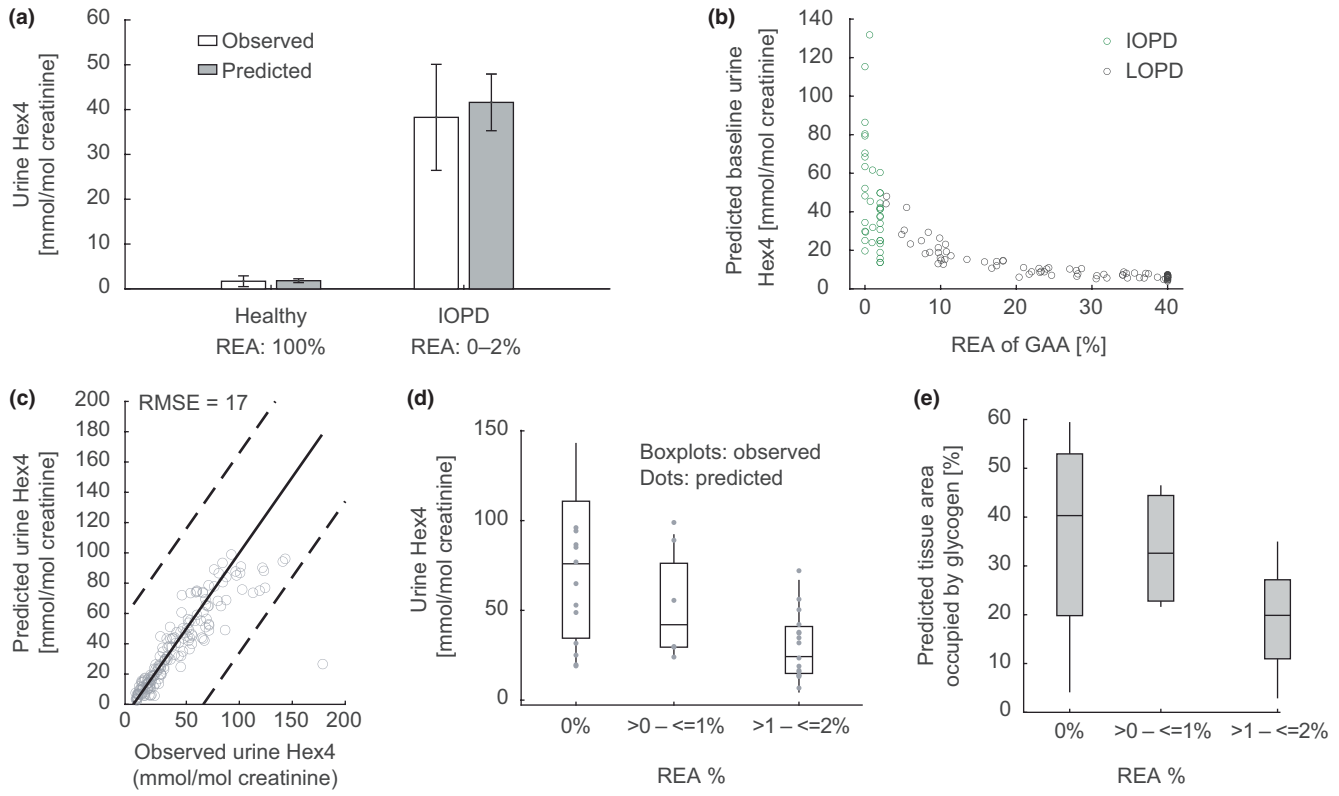

Figure 5 Development of IOPD digital twins. (**a**) Comparison of urine Hex4 predicted for IOPD patients less than a year old with clinical observations[21,24,28] (mean and standard deviation). (**b**) Relationship between digital twin-derived REA and corresponding baseline urine Hex4 values for patients enrolled in the COMET[27] or Mini-COMET[29] clinical studies. (**c**) GoF of urine Hex4 at baseline and week 49 for patients in Mini-COMET and treatment-naïve patients < 2 months of age in the Pompe Registry.[5] The solid and dashed lines indicate the identity +/− two standard deviations. Relationship between digital twin-derived REA values and (**d**) observed baseline urine Hex4 and (**e**) digital twin-derived skeletal muscle glycogen. GoF, goodness-of-fit; Hex4, hexose tetrasaccharide; IOPD, infantile-onset Pompe disease; REA, residual enzymatic activity; RMSE, Root Mean Square Error.

into the lysosome compared to treatment-responsive patients.[31,32] In addition to the REA, which defines the degree of enzymatic deficiency, a second time-invariant parameter representing the degree of progressive damage was implemented during the development of Mini-COMET digital twins. Lower values of this "responsiveness" parameter indicate greater extents of muscle damage.

Model calibrations captured with adequate precision the observed urine Hex4 data in Mini-COMET patients across all cohorts and treatment-naïve patients < 2 months in the Pompe Registry, as illustrated by the GoF plot (**Figure 5c**). As expected, optimized REA values were inversely related to baseline urine Hex4 and skeletal muscle glycogen with greater accumulation of urine Hex4 and glycogen in digital twins exhibiting lower REA values (greater enzyme deficiency) (**Figure 5d,e**).

Additionally, calibrated values of the "responsiveness" parameter had a significant, moderate correlation (Pearson's correlation coefficient = −0.5, $P < 0.05$) with baseline plasma creatine kinase (CK), a marker of muscle damage (**Figure S1**).[33] These "responsiveness" parameter values for each digital twin correlated with their respective patients' observed treatment duration. They also correlated with baseline CK values, which were not used during model development, and thus considered an independent validation supporting the interpretation of the "responsiveness" parameter as a proxy for the extent of ongoing muscle damage. These assessments suggest that the QSP model structure and parameter ranges utilized for the digital twins were appropriate to represent the heterogeneous disease presentation and therapeutic response of patients with IOPD in Mini-COMET.

To further evaluate model performance, an independent validation was performed using the efficacy data from IOPD patients not used during model development. In the Ph2/3 study, AGLU01602,[28] treatment-naïve IOPD patients < 6 months of age ($n = 18$) were treated with biweekly infusions of alglucosidase alfa at 20 or 40 mg/kg.[28] The majority of patients showed stable or decreased skeletal muscle glycogen levels and decreased urine Hex4, and this biomarker profile was associated with improved motor function. For this assessment, patients were classified as responders vs. minimal responders based on their observed motor function outcomes, tissue glycogen, and urine Hex4 response following 52 weeks of treatment.

A virtual population ($N = 12{,}000$) with representative demographics was constructed based on digital twins from Mini-COMET, sampling from REA, and "responsiveness" parameter ranges. Also, digital twins derived from treatment-naïve patients in the Pompe Registry < 2 months of age informed this virtual population. The virtual population was filtered to match the observed baseline urine Hex4 in AGLU01602 to perform a fair comparison. Following 52 weeks of simulated treatment, urine

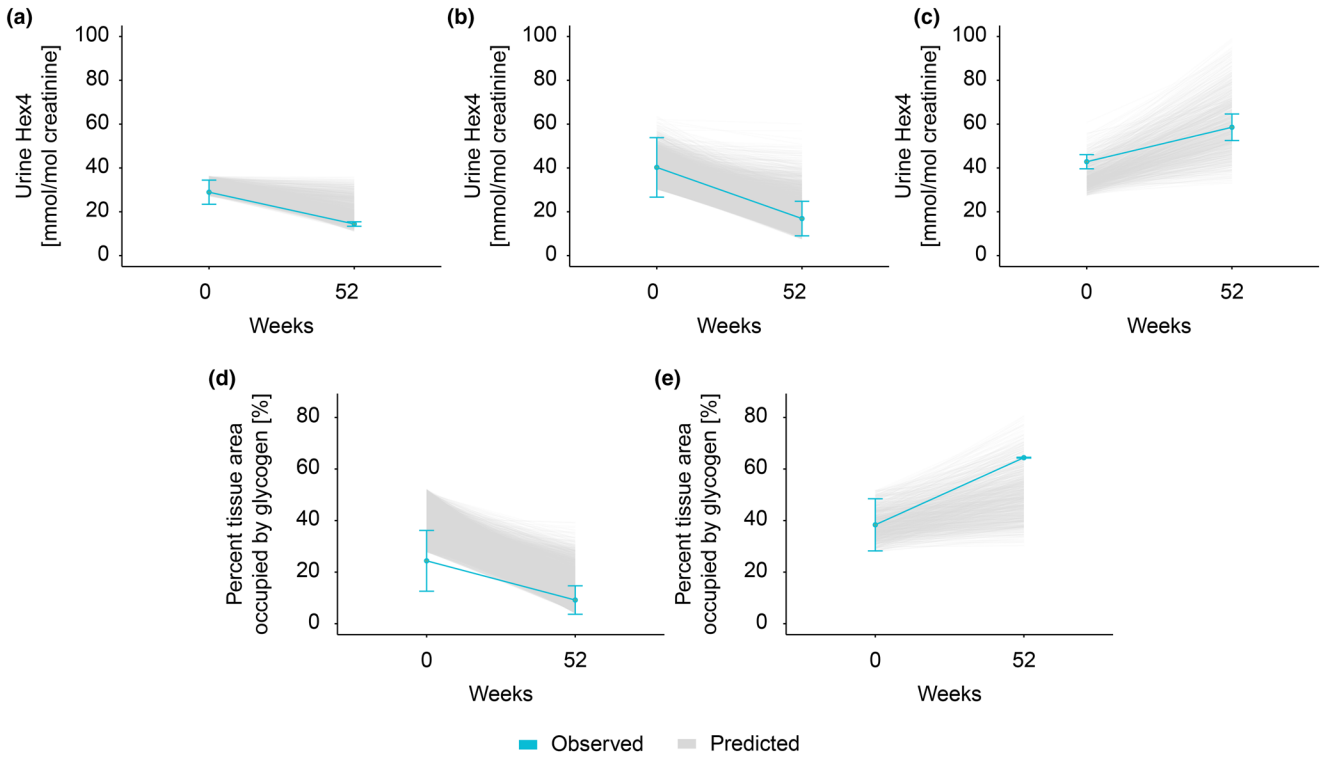

Figure 6 Validation of urine Hex4 representation in virtual IOPD population treated with alglucosidase alfa (gray envelope) through comparison with clinical data not used in model development[28] (blue lines indicating mean and standard deviation). Comparisons were performed with urine Hex4 observations for doses of (**a**) 20 mg/kg and (**b**) 40 mg/kg in responding patients (classified based on their observed motor function outcomes, tissue glycogen, and urine Hex4 response) and (**c**) for both doses in minimal responders Comparisons were performed with observations of % tissue area occupied by glycogen in skeletal muscle biopsies for both doses in (**d**) responders and (**e**) minimal responders. EOW, every other week; Hex4, hexose tetrasaccharide; IOPD, infantile-onset Pompe disease.

Hex4 outputs were compared to observed clinical data from patients for whom both baseline and week 52 measurements were available.

The model appropriately captured the urine Hex4 treatment response to alglucosidase alfa in responder patients treated with alglucosidase alfa at 20 or 40 mg/kg ($n = 10$) (**Figure 6a,b**). Due to the small number of minimal responders, patients from the 20 and 40 mg/kg cohorts ($n = 2$) were pooled, and the simulation of these patients showed adequate agreement with observed data (**Figure 6c**). The virtual population simulations also had adequate agreement with reported skeletal muscle glycogen changes in both cohorts (**Figure 6d,e**). Overall, these validation results further support the appropriateness of the glycogen and urine Hex4 representations in the QSP model and increase confidence in its application for *in silico* comparison of alglucosidase alfa and avalglucosidase alfa in IOPD patients.

In silico head-to-head comparison of alglucosidase alfa and avalglucosidase alfa in IOPD patients

IOPD is a heterogeneous disease, with wide variability reported in glycogen and urine Hex4 levels in untreated patients or patients showing clinical decline or sub-optimal clinical response to treatment.[28,29] To account for these differences in comparing the efficacy of alglucosidase alfa and avalglucosidase alfa, an *in silico* head-to-head comparison of three theoretical ERT regimens was performed using the Mini-COMET digital twins. These regimens were: (i) alglucosidase alfa monotherapy at 20 mg/kg; (ii) alglucosidase alfa at 40 mg/kg followed by a switch to avalglucosidase alfa at 40 mg/kg following 25 weeks of treatment; and (iii) avalglucosidase alfa monotherapy at 40 mg/kg. Each digital twin matches the demographic characteristics (age, sex, body weight) as the corresponding patient, as well as optimized REA and "responsiveness" parameter values based on their observed urine Hex4 dynamics. Each digital twin was simulated to their reported age at Mini-COMET baseline, with appropriate historical alglucosidase alfa dose and treatment duration, and was then treated with every theoretical regimen. Since each digital twin could be simulated for all regimens, not only the single arm in which the corresponding real patient was treated in Mini-COMET, a comparative assessment was possible across regimens that corrected for the observed baseline heterogeneity of the patients in each arm of Mini-COMET.

As shown in **Figure 7**, this analysis predicted that the enhanced reduction in urine Hex4 following avalglucosidase alfa treatment is attributable to enhanced skeletal muscle glycogen clearance. This analysis predicted that 40 mg/kg QOW avalglucosidase alfa treatment, whether for the duration of the whole study (97 weeks) or following a switch from alglucosidase alfa after 25 weeks, resulted in more pronounced reductions in urine Hex4 compared to alglucosidase alfa treatment.

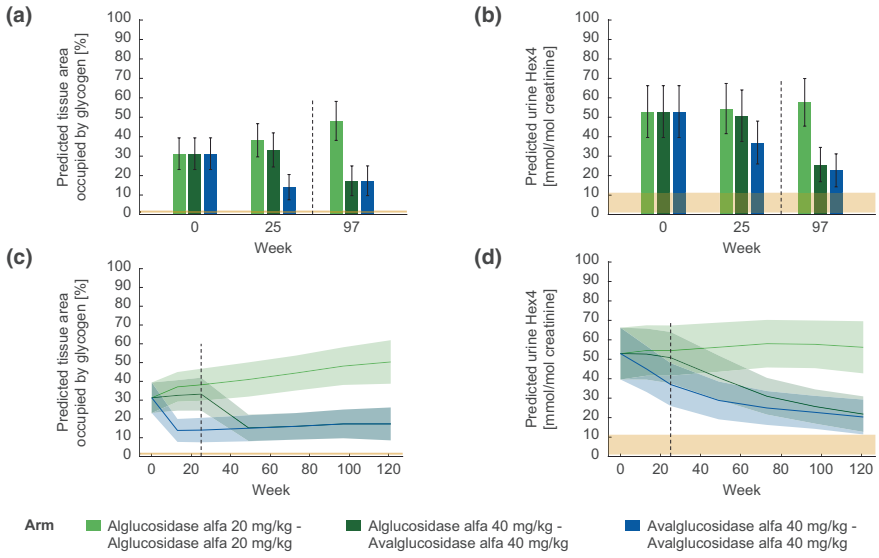

Figure 7 Comparison of theoretical monotherapy and switch regimens in the Mini-COMET digital twins in terms of (**a**) reduction of % skeletal muscle tissue area occupied by glycogen, compared to the reported range in healthy individuals (yellow shaded area)[18,46] and (**b**) urine Hex4, compared to the reported range in healthy individuals.[45] The corresponding time courses for each regimen are shown for (**c**) skeletal muscle glycogen and (**d**) urine Hex4. Hex4, hexose tetrasaccharide.

DISCUSSION

Drug development in rare diseases is characterized by unique challenges such as small patient number and high heterogeneity of disease presentation. Mechanism-based modeling provides a quantitative tool for integrating diverse data sources and supplementing clinical observations in these populations. In this study, a QSP model was developed to describe the key pathophysiology of Pompe disease. This study demonstrates that a single mechanistic model can be applied to describe the spectrum of Pompe disease in both adult and pediatric patients. The model adequately represents observed clinical and natural history data and was validated using independent datasets for both LOPD and IOPD populations.

While digital twin representations of LOPD patients and treatment-naïve IOPD patients required calibration of only the REA parameter, an additional time-invariant "responsiveness" parameter was introduced to describe IOPD patients enrolled in Mini-COMET. Per the enrollment criteria of Mini-COMET, these patients exhibited sub-optimal response following long-term alglucosidase alfa treatment. It was assumed that this response profile was possibly due to insufficient clearance of skeletal muscle glycogen by ERT, leading to continuous glycogen storage and muscle damage.[28,30,31] From a mechanistic perspective, this lack of responsiveness to ERT could be attributed to various factors, such as expression of the angiotensin-converting enzyme polymorphism,[34,35] autophagy buildup,[36,37] poor ERT tissue uptake,[38] variable expression of mannose-6-phosphate receptors,[39] or epigenetic factors.[40] Immunogenicity was also investigated as a possible causal factor, but a relationship could not be established based on available titer measurements. At present, insufficient data exists to assess the degree to which each of these factors contribute to individual patient responses, or the progression of muscle damage over time, so the "responsiveness" parameter was used to represent the cumulative effect. Further supporting the biological interpretability of this parameter, individually calibrated values had a moderate, significant correlation with reported baseline CK measurements in Mini-COMET (**Figure S1**). These data were not used during model development. Since CK is a proxy for muscle damage, this suggests that the "responsiveness" parameter is suitable for describing the trajectory of IOPD patients displaying clinical decline or sub-optimal clinical response.

Calibrated REA and "responsiveness" parameters, together with reported demographic data, were used to implement the QSP-based digital twin analysis. This approach resulted in a virtual representation of each IOPD patient enrolled in Mini-COMET that recapitulated their observed individual efficacy data with adequate precision. The glycogen and biomarker response of each digital twin could then be simulated following various dosing regimens of alglucosidase alfa or avalglucosidase alfa. Since the same digital IOPD twin population was simulated for all regimens, this compensated for the patient heterogeneity observed at baseline in Mini-COMET. Overall, the digital twin analysis confirms clinical observations that avalglucosidase alfa treatment leads to a greater reduction in urine Hex4, and further supplements clinical observations by supporting the interpretation that the enhanced reduction in urine Hex4 observed in the avalglucosidase alfa arms is attributable to greater tissue glycogen clearance. Despite having no available skeletal muscle biopsies from avalglucosidase alfa-treated patients with IOPD, the validated glycogen representation in the model suggests further debulking of glycogen in patients treated with avalglucosidase alfa, even those that had reached stable urine Hex4.

The digital twins approach enables the supplementation of clinical trial data in rare indications like IOPD, which are often characterized by substantial phenotypic variability. Because clinical trials in these indications are necessarily small, this patient heterogeneity can impede the interpretation of the clinical response. QSP-based digital twin analyses provide a

mechanism- and patient data-driven approach to help address this challenge. In the current study, digital twins support the head-to-head comparisons of alternative drug regimens, controlling for any baseline differences in each cohort that may affect the interpretation of the drug response. This approach can be further refined by incorporating emerging data types, including -omics data[41,42] and imaging. In particular, novel imaging modalities that are being developed to non-invasively measure skeletal muscle damage[43] and glycogen levels[44] may help to improve the generation of digital twins of Pompe patients and to link the tissue glycogen simulated by the QSP model to commonly used clinical or functional endpoints like the 6-minute walk test.

The Pompe QSP model can be deployed in multiple stages of the R&D lifecycle. In addition to gaining insight into potential biological drivers of clinical response, other applications of the QSP model include the exploration of alternative drug modalities, combination with other treatments, and first-in-human dose selection for other novel drugs for Pompe disease. This digital twin QSP approach can also be applied in other rare diseases to enable more effective and efficient drug development.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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AUTHOR CONTRIBUTIONS

C.K., M.T., and S.Z. wrote the manuscript. C.K., M.T., S.B., K.G., H.G., P.H.G., J.L.B., M.F., C.O.-R., A.Z., K.A.H., and S.Z. designed the research. C.K., M.T., S.B., and S.Z. performed the research and analyzed the data.

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CONFLICTS OF INTEREST

C.K., M.T., S.Z., K.G., J.L.B., M.F., C.O.-R., A.Z., and K.A.H. are employees of Sanofi and may own stocks or stock options. H.G. and P.H.v.d.G. are employees of Certara and may own stock options. S.B. was an employee of Certara at the time this work was performed and may own stock options. As the Editor-in-Chief of *Clinical Pharmacology and Therapeutics*, Piet H. van der Graaf was not involved in the review or decision process for this paper.

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