Quantitative Relationship Between Tominersen Concentrations in Cerebrospinal Fluid and Biomarker Changes in Huntington's Disease Patients

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1. What is already known about this subject

- Huntington's disease (HD) is a fatal neurodegenerative disease with no available disease-modifying treatments.
- Tominersen, an antisense oligonucleotide, aims to slow HD by reducing the mutant huntingtin (mHTT) protein.
- While its Phase 3 study did not meet primary clinical endpoints, it confirmed that tominersen reduces mHTT levels in cerebrospinal fluid (CSF).

2. What this study adds:

- The developed population pharmacokinetic/pharmacodynamic model quantitatively links tominersen concentrations to mHTT protein reduction in CSF.
- Lower tominersen exposures avoid the transient elevations in some CSF biomarkers observed at high exposures.
- The favorable biomarker profiles in the lowest exposure group provide a data-driven rationale for investigating lower tominersen doses.

ABSTRACT (248 words)

Aim: Intrathecally administered antisense oligonucleotide tominersen aims to slow Huntington's disease progression by lowering mutant huntingtin protein (mHTT) levels. This study used non-linear mixed effects population pharmacokinetic and pharmacodynamic (PKPD) modeling to characterize the relationship between tominersen concentration in cerebrospinal fluid (CSF) and CSF mHTT reduction. Additionally, the relationship between tominersen CSF exposure and changes in other CSF biomarkers was investigated to understand tominersen's pharmacodynamic profile. Finally, PKPD model simulations were conducted to inform the dose selection in the GENERATION HD2 study (GEN-HD2).

Methods: Data from four clinical studies, including 915 participants receiving placebo or tominersen doses (30–120 mg) every 4, 8, or 16 weeks for up to 25 months, were used to develop the PKPD model. The model was utilized to predict tominersen CSF exposure metrics for individual patients in the GENERATION HD1 study for the exposure-response (ER) analysis and to simulate the PK and PD profiles for lower doses.

Results: An indirect response model described the relationship between tominersen CSF concentration and mHTT reduction, estimating a half-maximal inhibitory concentration (IC_{50}) of 4.18 ng/mL. The ER analysis revealed that the highest exposure quartile showed a 54% mHTT reduction at steady state and transient elevations in biomarkers of neuroinjury and inflammation. In contrast, the lowest exposure quartile had a 24% mHTT reduction and a favorable biomarker profile.

Conclusions: The PKPD model quantitatively confirms the relationship between tominersen exposure and CSF mHTT lowering. The ER analysis suggests that lower tominersen exposure levels may offer a better benefit-risk profile.

INTRODUCTION

Huntington's disease (HD) is a rare, autosomal dominant monogenic disorder characterized by progressive cognitive and motor impairment, as well as behavioral and metabolic alterations. [1-3] Typically, the onset of symptoms occurs during adulthood, with an average survival of approximately 15 years following the diagnosis of clinical motor onset.[4]

HD is caused by a cytosine-adenine-guanine (CAG) trinucleotide repeat expansion in the huntingtin (*HTT*) gene located on the 4th chromosome.[5] This expansion leads to the production of a toxic mutant huntingtin (mHTT) protein, which disrupts a wide range of normal physiological functions.[2,3,6] Given the monogenic nature of HD, lowering mHTT protein levels is currently a central therapeutic strategy under investigation for mitigating the pathogenesis of HD. At present, there are no disease-modifying treatments that can slow or halt the progression of HD or delay the onset of its clinical symptoms.[2]

Recently, research has focused on fluid biomarkers in cerebrospinal fluid (CSF) that reflect the underlying neuropathological changes in HD. Key biomarkers include neurofilament light chain (NfL) and total Tau, which are general indicators of neuronal injury and death[7-9]. Phosphorylated Tau 181 (pTau-181) is a more specific marker for aggregated tau pathology.[9] Additionally, markers of glial cell activity and neuroinflammation are highly relevant in HD. Glial fibrillary acidic protein (GFAP) is a marker of astrocytic reactivity[12] while chitinase-3-like protein 1 (YKL-40) indicates neuroinflammation.[13] Observational studies in HD have shown that CSF levels of several of these markers are elevated in CAG repeat expansion carriers and are associated with clinical measures of disease severity.[14-16]

Tominersen is an investigational chimeric 2′-O-(2-methoxyethyl) (2-MOE) modified antisense oligonucleotide (ASO) designed to selectively reduce the expression of human HTT messenger RNA (mRNA). The drug works by binding to its target HTT mRNA, and this binding action subsequently triggers the RNase H1-mediated degradation of the mRNA. By destroying the mRNA, tominersen effectively prevents the translation and production of the huntingtin protein. [17] To date, it has been investigated in individuals with manifest HD in five clinical studies (Phase I/IIa study, NCT02519036 (CS1); open-label extension of the Phase I/IIa study, NCT03342053 (CS2); GENERATION HD1, NCT03761849 (GEN-HD1); GEN-PEAK, NCT04000594 and GEN-EXTEND, NCT03842969).[17-21] These studies investigated doses ranging from 10 mg to 120 mg, administered for up to 25 months. The CS1 study was the first to demonstrate a dose-dependent reduction in CSF levels of mHTT in humans.[17] In the GEN-HD1 trial, the largest clinical study conducted in Huntington's Disease to date, the primary clinical endpoint, measured by the composite Unified Huntington's Disease Rating Scale (cUHDRS), showed that the tominersen group receiving doses every 8 weeks (Q8W) performed worse than the placebo group. In contrast, the group receiving doses every 16 weeks (Q16W) had outcomes comparable to the placebo. A post-hoc analysis of GEN-HD1 identified the potential for treatment benefits in younger individuals with a lower disease burden at lower tominersen CSF exposure levels.[22] Further analysis of the data from the clinical studies following tominersen administration may provide important insights into the HTT-lowering approaches to treat HD.

The tominersen population pharmacokinetic (popPK) model, which described both plasma and CSF PK simultaneously, has been reported.[24] The current work presents a population pharmacokinetic-pharmacodynamic (PKPD) model that leverages the established popPK model to quantitatively characterize the relationship between tominersen concentration in CSF and the reduction of mHTT in the CSF. Furthermore, we conducted an exposure-response (ER) analysis using the developed PKPD model to explore the relationship between tominersen CSF exposure and changes in CSF biomarkers following tominersen treatment. By integrating CSF biomarkers into the ER analysis, we aim to develop a comprehensive understanding of tominersen's pharmacodynamic profile. This approach will provide valuable insights into the risks and potential benefits associated with tominersen CSF exposure, enhancing our understanding of its therapeutic potential. Finally, we conducted simulations using the developed PKPD model to inform dose selection for GENERATION HD2 (GEN-HD2).

METHODS

Subject and study design

The studies included in the analysis were conducted in accordance with the principles of the Declaration of Helsinki. Each study received approval from the appropriate institutional review boards, local ethics committees, and regulatory agencies at all participating sites. All patients provided written informed consent.

PK and CSF mHTT data collected in individuals with manifest HD from the four clinical studies - CS2, GEN-HD1, GEN-PEAK and GEN-EXTEND were pooled to develop a PKPD model.[18-21] It should be noted that the term 'manifest HD' reflects the diagnostic criteria used during study conduct, prior to the publication of the Huntington's Disease Integrated Staging System (HD-ISS).[25] The GEN-EXTEND study is an open-label trial that enrolled patients who had completed any other clinical study in the tominersen program.

The subsequent ER analysis focused on participants from the GEN-HD1, which provided the most comprehensive long-term biomarker data. The ER analysis included participants from the placebo group who had at least one biomarker measurement, and participants from the active dose groups (120 mg Q8W or Q16W) who had both at least one biomarker measurement and one measurable tominersen concentration in CSF.

Patient numbers, CSF mHTT data, dose and dosing frequency, study length and CSF biomarker sampling time points are provided in **Table 1**.

Bioanalytical methods

Tominersen CSF PK

Tominersen concentrations in CSF were determined using a validated hybridization electrochemiluminescence assay. The lower limit of quantification (LLOQ) was 0.1 ng/mL in CSF.[24]

Pharmacodynamic measurements

The mHTT protein concentrations in CSF were measured using a bead-based ligand binding assay with the 2B7 antibody for capture and the MW1 antibody for detection on the SMCxPRO™ platform (Merck).[26] The LLOQ was 25.08 fM.
All neuronal and glial biomarkers of neurodegeneration and inflammation were measured as part of the NeuroToolKit, a portfolio of immuno assays available on the fully automated Elecsys® platform.

Population PKPD model development

Structural and stochastic model development

Structural model development

Non-linear mixed effects analysis was performed to develop the PKPD model. The PKPD model was developed by leveraging the previously developed popPK model using the individual empirical Bayes estimates (EBEs) of PK parameters for patients in the tominersen treatment groups.[24] The summary of the previously developed PK model as well as the estimated PK parameters are provided in **Supplementary materials**. An indirect-response model was chosen to describe the turnover of mHTT protein in CSF. This structure is mechanistically plausible, as tominersen prevents translation of HTT protein by binding to HTT mRNA. The model assumes that tominersen concentration in the CSF inhibits the zero-order production rate constant (k_{IN}) of CSF mHTT. The elimination rate constant of CSF mHTT (k_{OUT}) was calculated as k_{IN} divided by baseline CSF mHTT concentration. The drug effect was included as a negative effect of individually predicted tominersen concentrations in CSF on k_{IN}. Schematic representation of the PKPD model is provided in **Supplementary materials**.

Interindividual variability

Interindividual variability (IIV) was evaluated on all parameters by using an exponential form (Equation 1):

$$Pi = TVP \times e^{\eta i} \qquad (1)$$

where TVP is the typical value of the parameter P, P_i is the individual value of the parameter and ηi is a normally distributed random variable with mean 0 and standard deviation ω.

Residual error

An additive residual error model was used on the log-transformed mHTT in CSF, which corresponds approximately to a proportional error on untransformed data.

Handling of the samples below the LLOQ

A large number of post-dose CSF mHTT observations fell below the LLOQ of 25.08 fM, particularly in the higher exposure groups (**Table 1**). To mitigate the bias that can arise from either discarding these data or using simple imputation methods, a likelihood-based approach (the M3 method, in which the likelihood of an observation being below the LLOQ is modeled) was implemented in NONMEM.[27,28]

Covariate analysis

The covariate analysis was a formal two-step process. First, covariates with strong a priori scientific rationale were evaluated manually by incorporating them into the structural model based on a statistically significant drop in the objective function value (OFV) and improved goodness-of-fit (GOF) plots. The mHTT assay is known to depend on the CAG repeat length[26,29], and therefore, the effect of CAG repeat length on CSF mHTT baseline was included in the base model and was not further evaluated in the stepwise procedure.

Second, a broader systematic screening of all potential covariates was performed using the automated Stepwise Covariate Modeling (SCM) procedure in PsN.[30] In this analysis, adaptive scope reduction (ASR) was added to the default SCM algorithm to make the covariate search

more efficient.[31] To protect the model from spurious associations, stringent p-value criteria were employed. A forward inclusion criterion of $p < 0.01$ and a backward elimination criterion of $p < 0.001$ were used, which retained only the most robust and impactful covariates. A covariate identified as statistically significant by SCM was only kept in the final model if it also passed a pre-defined threshold for clinical relevance: causing at least a 10% change in the parameter between the 5th and 95th percentiles of the covariate's distribution. The following covariates at baseline were evaluated on baseline CSF mHTT and IC_{50}: age, CAG-Age-Product (CAP) score, caudate volume, ventricular volume, whole brain volume, NfL levels in CSF and cUHDRS.

Consistent mathematical functions were applied to model covariate-parameter relationships across both the manual and the SCM evaluation steps. Continuous covariates were modeled using a power function, while categorical covariates were modeled using a proportional model (Equation 2 and 3):

$$Pi = TVP \times \left(\frac{COV_i}{COV_{median}}\right)^{\vartheta_{cov}} \quad (2)$$

$$Pi = TVP \times (1 + \vartheta_{cov}) \quad (3)$$

where P_i is the individual parameter estimate, TVP is the typical value of the parameter, COV_i is the individual's covariate value, COV_{median} is the median population covariate value, and θ_{COV} is the estimated parameter for the covariate effect.

Model selection

The performance of a model, and selection between competing models, was based on statistical and graphical assessments including the inspection of GOF and changes in the OFV provided by NONMEM. The differences in OFVs (ΔOFVs) are nominally χ^2 distributed and a difference of 3.84 corresponds to approximately a p-value of < 0.05 for one degree of freedom, provided that the models are nested.

Model evaluation

In addition to the model selection criteria described above, simulation based diagnostics such as visual predictive check (VPC) were used to evaluate the predictive performance of the final model.[32]

Exposure-response analysis

Individual CSF PK exposure and CSF mHTT reduction endpoints

The developed PKPD model was used to compute individual average tominersen CSF concentration at steady state ($C_{av,CSF,SS}$) and to compute individual average mHTT reduction in CSF at steady state ($mHTT_{av,SS}$) using estimated individual PK and PD parameters and actual dosing history.

Graphical examination

The graphical exposure-response analysis was performed by splitting the patients in the active dose groups (120mg Q8W or Q16W) in GEN-HD1 into four equally sized groups based on the

computed $C_{av,CSF,SS}$ and $mHTT_{av,SS}$. The relationship between the computed individual exposure metrics and the percent change from baseline for CSF fluid biomarkers, including NfL, YKL-40, total Tau, pTau-181, GFAP and total protein were graphically examined.

Simulations

The final PKPD model was utilized to simulate $C_{av,CSF,SS}$ and the time-concentration profile of CSF mHTT reduction for 60 and 100 mg Q16W in patients aged 25–50 years with a CAP score of 400–500, which is aligned with inclusion/exclusion criteria for GEN-HD2.[23]

Software

The PKPD model development and simulations were performed using NONMEM version 7.4.4.[33] NONMEM runs were performed using the gfortran compiler, version 4.4.6. Parameter estimation was performed using the Laplace method in NONMEM.

Data management and a graphical analysis including ER analysis were performed using R version 3.5.3 or above.[34] SCM and visual predictive checks were performed using Perl-speaks-NONMEM (PsN) version 4.9.0.[35,36]

RESULTS

Analysis dataset for PKPD modeling

A total of 3613 quantifiable mHTT observations in CSF and 529 observations below the LLOQ from 915 individuals receiving placebo or tominersen doses ranging from 30–120 mg every 4 weeks (Q4W), Q8W or Q16W for up to 25 months were included in the data set for model development. The baseline covariates are summarized in **Supplementary materials**. **Figure 1** presents the longitudinal percent changes in CSF mHTT, demonstrating a clear reduction that is dependent on dosing frequency and, consequently, tominersen exposure.

Population PKPD model

An indirect response model, where tominersen concentrations in CSF inhibit the production rate of CSF mHTT through an I_{max} model described the observed CSF mHTT data well. The parameter estimates of the final model are shown in **Table 2**.

CAG repeat number, CSF NfL and ventricular volume were relevant covariates for baseline CSF mHTT level. The final model estimated that baseline mHTT was higher with an increasing CAG repeat number (110% higher at the 95th percentile vs. the 5th percentile of CAG), higher with increasing baseline NfL (44% higher at the 95th percentile vs. the 5th percentile of NfL), and lower with increasing ventricle volume (19% lower at the 95th percentile vs. the 5th percentile of ventricle volume). None of the tested covariates (age, whole brain volume, caudate volume, ventricular volume, CAP, CAG, NfL or cUHDRS) was found to be statistically significant for IC_{50}. The correlation between baseline CSF mHTT level and IC_{50} was tested using an omega block but was not found to be statistically significant. The LLOQ (25.08 fM) was high in relation to the baseline CSF mHTT levels (median value of baseline CSF mHTT levels was 62.9 fM) and approximately 30% of samples in Q4W 120 mg group were below the LLOQ after tominersen administration. Therefore, the I_{max} was not estimated, but fixed to 1, which is the theoretical maximum inhibition of the CSF mHTT production. In general, parameters were estimated with high precision (relative standard error (RSE) < 8%) except for the impact of ventricle volume on the baseline CSF mHTT (RSE was approximately 17%). IIV was supported by the data and included on baseline CSF mHTT and IC_{50}, with estimated coefficients of variation of 34.0% and 69.3%, respectively.

The GOF plots showed adequate agreement between predicted and observed CSF mHTT, without any significant trends (**Supplementary materials**). The prediction corrected VPC (pcVPC) for CSF mHTT versus time confirmed the good predictive performance of the final model to capture the CSF mHTT profile, individual variability as well as the percentage of the samples below the LLOQ (**Figure 2**).

Predicted individual exposure parameters

A total of 766 patients were included in the ER analysis. The quartile groups of $C_{av,CSF,SS}$ in GEN-HD1 were defined as follows: Q1 ranged from 1.45 to ≤2.40 µg/mL; Q2 ranged from >2.40 to ≤3.28 µg/mL; Q3 ranged from >3.28 to ≤4.73 µg/mL; and Q4 ranged from >4.73 to ≤7.36

μg/mL from the lowest to highest exposure group. The quartile groups of $mHTT_{av,SS}$ in GEN-HD1 were defined as follows: Q1 ranged from 14.0 to ≤28.4%; Q2 ranged from >28.4 to ≤37.1%; Q3 ranged from >37.1 to ≤47.8%; and Q4 ranged from >47.8 to ≤72.2% from the lowest to highest CSF mHTT reduction group. 98% of the patients in the Q16W group fell into either the Q1 or Q2 group of $C_{av,CSF,SS}$, while 97% of the patients in the Q8W group fell into either the Q3 or Q4 group of $C_{av,CSF,SS}$ (**Supplementary materials**). A similar trend was observed in the distribution of $mHTT_{av,SS}$, where 82% of patients in the Q16W group were classified into either the Q1 or Q2 group, while 81% of patients in the Q8W group were classified into either the Q3 or Q4 group. There was a larger variability in $mHTT_{av,SS}$, therefore, $C_{av,CSF,SS}$ was used for the ER analysis rather than $mHTT_{av,SS}$.

Exposure-response analysis

Figure 3A displays the median longitudinal changes of CSF fluid biomarkers from baseline, including CSF mHTT, NfL, YKL-40, total Tau, pTau-181, GFAP, and total protein across different exposure quartile groups and placebo in GEN-HD1. As anticipated from the PKPD modeling, exposure-dependent reductions in CSF mHTT were observed. At steady state, the median mHTT reduction was 54% in the highest exposure quartile and 24% in the lowest exposure quartile.

A transient exposure-dependent elevation in NfL, total Tau, pTau-181 and YKL-40 was observed around 13 and 21 weeks after the start of tominersen treatment. These transient increases were most evident in the highest exposure group and were absent in the lowest exposure group. Similarly, an increase in GFAP was most pronounced in the highest exposure group but decreased in an exposure-dependent manner.

Figure 3B illustrates the median longitudinal changes in the CSF biomarkers from baseline, along with their variability, for both the placebo group and the lowest exposure group in the GEN-HD1. In the lowest exposure group, at the median level at week 69, total Tau and pTau-181 decreased compared to placebo. NfL and YKL-40 showed trends below placebo levels, while GFAP and total protein were comparable to those of the placebo group. The plots for other exposure quartiles are provided in **Supplementary Materials**.

Prediction of tominersen CSF PK and CSF mHTT reduction to inform dose selection for GEN-HD2

Model predicted $C_{av,CSF,SS}$ with doses of 60 and 100 mg Q16W, which have previously not been administered to patients, are shown in **Figure 4A**. These predictions suggest that the CSF concentration following Q16W administration of 100 mg tominersen will be in the range of the lowest exposure group in GEN-HD1 (Q1). With a 60 mg Q16W dosing regimen, the CSF exposures are predicted to be below the range of concentrations explored in GEN-HD1. The predicted time-concentration profiles of CSF mHTT reduction are illustrated in **Figure 4B**. The predicted reduction in CSF mHTT at steady state is expected to range from a minimum of 11% to a maximum of 21% over the dosing interval with the 60 mg Q16W dosing regimen. With the

100 mg Q16W dosing regimen, the CSF mHTT reduction is expected to range from a minimum of 15% to a maximum of 28%.

DISCUSSION

A PKPD model was developed using pooled data from four clinical studies where placebo or tominersen doses ranging from 30 to 120 mg were administered intrathecally at frequencies of Q4W, Q8W and Q16W. In total, 3613 quantifiable mHTT observations in CSF together with 529 samples below the LLOQ from 915 people with HD were included in the analysis. The PKPD model adequately described CSF mHTT profiles after administration of tominersen or placebo. The ER analyses indicated that increases in NfL, total Tau, pTau-181, YKL-40 and GFAP observed in the highest exposure group were avoided in the lowest exposure group, while anticipated exposure-dependent reductions in CSF mHTT were observed. Furthermore, the lowest exposure group showed trends below placebo for NfL, YKL-40, total Tau and pTau-181, while GFAP and total protein were comparable to placebo at week 69. The anticipated CSF mHTT reduction in combination with a favorable biomarker response profile suggests that future clinical studies should focus on lower tominersen exposure levels to optimize the benefit-risk profile.

The CSF mHTT was adequately described by an indirect-response model, where the production of CSF mHTT was inhibited by tominersen concentrations in CSF. Model diagnostics for the final PKPD model indicated a robust predictive performance. The analysis demonstrated evidence of target engagement. IC_{50} was 4.18 ng/mL, corresponding to the CSF concentration at approximately 4 weeks after tominersen IT administration of 120 mg Q4W at steady state.[24] The observed mHTT concentration profiles in CSF suggested a very slow turnover rate. Based on the estimated parameters in the PKPD model, turnover half-life of CSF mHTT was approximately 1 month. This calculated slow turnover rate supports a dosing regimen with infrequent intrathecal administration of Q16W.

The ER analyses revealed a clear association between tominersen exposure and biomarker dynamics. Higher tominersen exposure was associated with elevations in a panel of CSF biomarkers such as NfL, total Tau, pTau-181, YKL-40, and GFAP. There may be an association between these biomarker elevations and the clinical outcomes observed in the 120 mg Q8W dosing group of the GEN-HD1 study; the majority (97%) of these participants were in the higher exposure quartiles and, as reported previously, experienced worse outcomes on the cUHDRS than the placebo group.[22]

Conversely, these biomarker elevations were mitigated at lower exposure levels, a finding that corresponds with the 120 mg Q16W group, where 98% of participants were in the lower exposure quartiles and had clinical outcomes comparable to placebo.[22] Furthermore, the favorable biomarker profile in the lowest exposure group, which achieved a median mHTT reduction of 24% at steady state, is mirrored by the findings of a post-hoc exploratory analysis of GEN-HD1. This post-hoc analysis showed that point estimates of all UHDRS endpoints were consistently in a favorable direction compared with placebo in younger individuals with a lower disease burden at lower tominersen CSF exposure, although significance testing was not undertaken.[22] Taken together, these observations allow for the hypothesis that reducing

tominersen exposure could avoid the unfavorable biological signals seen at higher exposures and may lead to beneficial clinical effects.

Currently GEN-HD2 is ongoing with 2 dose levels of 60 mg and 100 mg Q16W.[23] PKPD model predictions for GEN-HD2 suggested that a 100 mg Q16W dosing regimen is suitable to target a CSF exposure equivalent to the lowest exposure group in GEN-HD1 and is expected to result in approximately 15-28% CSF mHTT reductions. The 60 mg Q16W dosing regimen was selected to explore lower tominersen CSF exposures, which were not explored in GEN-HD1, with expected CSF mHTT reductions of approximately 11-21%. We consider 100 mg Q16W appropriate to test the hypotheses suggested by the GEN-HD1 post-hoc analysis, while 60 mg Q16W will allow further characterization of the lower limit of therapeutic range of CSF exposure.

In conclusion, the developed PKPD model was able to describe CSF mHTT profiles after administration of tominersen and placebo and provided insights into CSF mHTT dynamics. The ER analysis using individual tominersen CSF PK exposure supports investigating lower tominersen exposure. The model and ER analysis enabled a data-driven interpretation of the clinical data and supported decision-making on the clinical development of tominersen.

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Conflict of Interest

Y.Y., H.E.S.B., P.G., D.J.H., P.M., and P.S.D. are current employees of and own stock in F Hoffmann-La Roche. M.B. is an employee of and owns stock in Pharmetheus AB. K.A., B.R.L., B.G.L., S.T., and E.J.W. are members of Scientific Advisory Board for GENERATION HD1 study.

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Author’s Contribution

Y.Y., H.E.S.B., M.B., D.J.H., P.M. and P.S.D. wrote the manuscript. Y.Y., and M.B. performed the research and H.E.S.B. and P.S.D. reviewed the analyses. Y.Y. and P.S.D. designed the research. P.G. prepared the dataset for the research. K.A., B.R.L., B.G.L., S.T., and E.J.W. designed tominersen clinical studies.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1. Overview of clinical data

Study	Dose regimen	N of IT injection / Length of the study	CSF mHTT protein sampling time points	N of subjects for CSF mHTT	total N of CSF mHTT observations (N of samples < LLOQ)	CSF biomarker sampling time points
OLE of the Phase I/IIa study (NCT03342053)(CS2)	120 mg Q4W	15 months	Before each dose	23	237 (75)	-
	120 mg Q8W			23	143 (38)	
GEN-PEAK (NCT04000594)	30 mg Q4W	2 doses	Pre-dose; at 2, 4, 8, 12, 16, 24, 36, 48, 60, and 72 hr after the first dose; before the second dose; on Days 43, 71, and 127; and at 6 months after last dose	4	10 (0)	-
	60 mg Q4W			4	31 (0)	
	120 mg Q4W			4	73 (0)	
GENERATION HD1[a] (NCT03761849)	Placebo	97 weeks	Pre-dose at Weeks 1, 5, and thereafter Q8W (Pre-dose at Weeks 1, 5, 13, 21, 37, (53) and 69)	281	1133 (41)	Pre-dose at Weeks 1, 5, 21, 37, (53) and 69
	120 mg Q8W			253	923 (189)	
	120 mg Q16W			247	923 (107)	
GEN-EXTEND[b] (NCT03842969)	120 mg Q4W	Up to 6 years	One sample at inclusion and before each dose, and at end of study	16	86 (14)	-
	120 mg Q8W			23	181 (20)	
	120 mg Q8W (no loading)			54	266 (39)	
	120 mg Q16W			1	3 (0)	
	120mg Q16W (no loading)			54	133 (6)	

CSF, cerebrospinal fluid; mHTT, mutant huntingtin; hr, hour; IT, intrathecal; LLOQ, lower limit of quantification; N, number; Q4W, every 4 weeks; Q8W, every 8 weeks; Q16W, every 16 weeks.

a: Protocol version 3, which enrolled patients with a dose regimen of 120 mg Q4W/Q8W or placebo, is combined with Protocol version 5, which enrolled patients with a dose regimen of 120 mg Q8W/Q16W or placebo.

b: initial dose regimen. Some individuals participating in the GEN-EXTEND open-label extension study were rolled over from the GEN-HD1 study.

Table 2. Parameter estimates of the final PKPD model

Final model	Unit	Typical value	RSE (%)	SHR (%)
Baseline mHTT	fM	62.9	1.49	
k_{in}	/h	0.0553	7.81	
I_{max}		1 (FIX)	-	
IC_{50}	ng/mL	4.18	5.66	
CAG_Baseline mHTT		0.0661	-	
NfL_Baseline mHTT		0.0000725	7.75	
Ventricle vol._Baseline mHTT		-0.00357	17.3	
Interindividual variability (IIV)[a]				
IIV on Baseline mHTT	%	34	2.99	13.8
IIV on IC_{50}	%	69.3	7.67	53.2
IIV RUV		33.8	5.96	44.6
Residual unexplained variability (RUV)[a]				
RUV_proportional	%	30.5	1.11	22.7

Shrinkage was calculated using the standard deviation-based method: $[1-SD(\eta_i)/\omega]\times100$, where $SD(\eta_i)$ is the standard deviation of the individual empirical Bayes estimates of the random effect, and ω is the model-estimated standard deviation of the interindividual variability for that parameter.
The PD parameter estimation was performed using the Laplace method in NONMEM.

a: Interindividual variability and residual unexplained variability are expressed as coefficient of variation and in % of the parameter estimate.
mHTT: mutant huntingtin protein; k_{in}: zero-order production rate constant; I_{max}: maximum inhibition; IC_{50}: concentration at half maximum inhibition; vol: volume; RSE: relative standard error; IIV: interindividual variability; RUV: residual unexplained variability; SHR: shrinkage

Figures

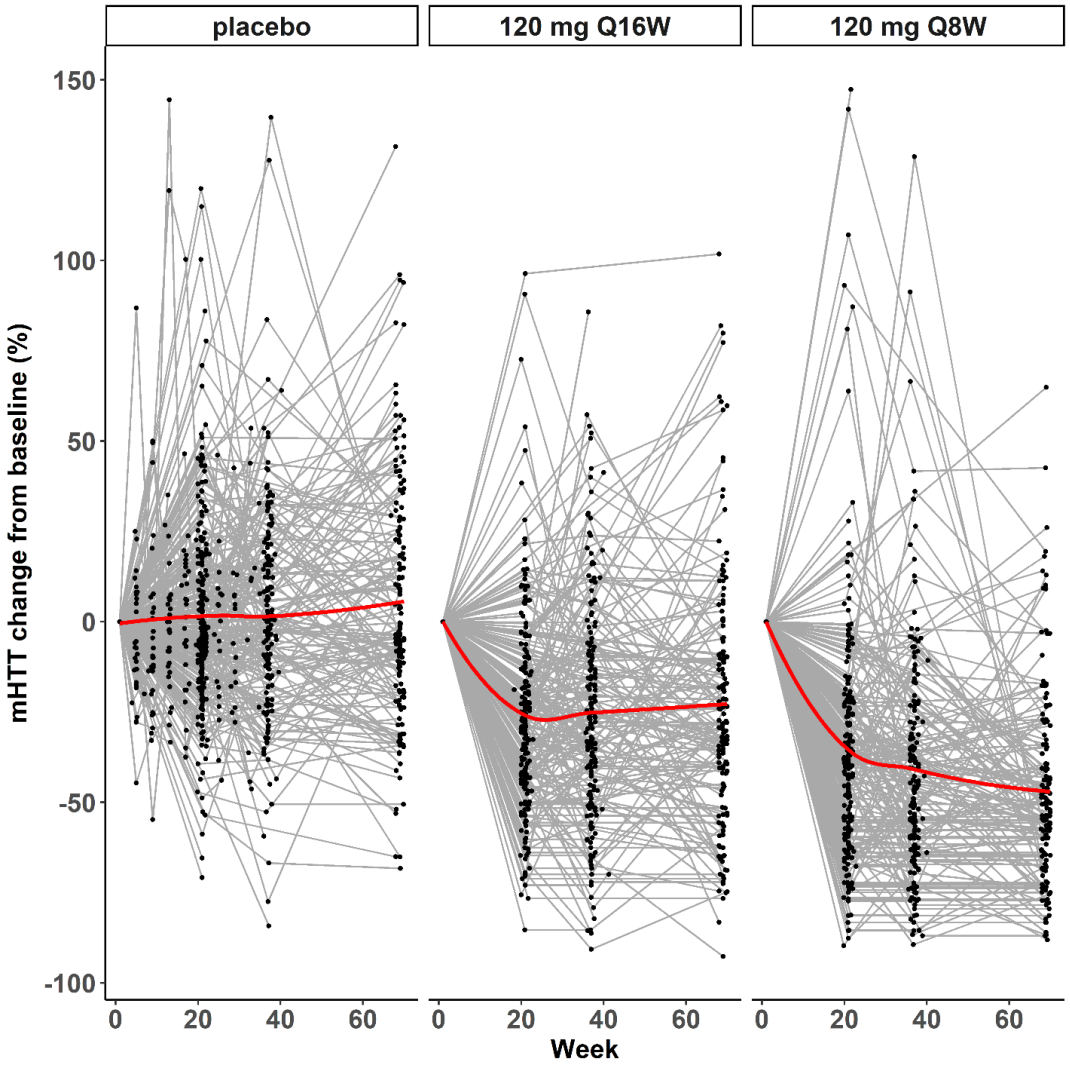

Figure 1
Observed percent changes in CSF mHTT from baseline for placebo, Q8W and Q16W at 120 mg. Black circles represent the observed concentrations, while individual profiles are shown with grey lines. Smooth trend lines are shown in red. Patients without baseline CSF mHTT data (no samples or samples below the LLOQ) were excluded. Post- treatment samples below the LLOQ are also excluded.
Q4W data is excluded due to limited data and a short treatment period with multiple doses.

CSF, cerebrospinal fluid; mHTT, mutant huntingtin protein; Q4W, every 4 weeks; Q8W, every 8 weeks; Q16W, every 16 weeks; LLOQ, lower limit of quantification.

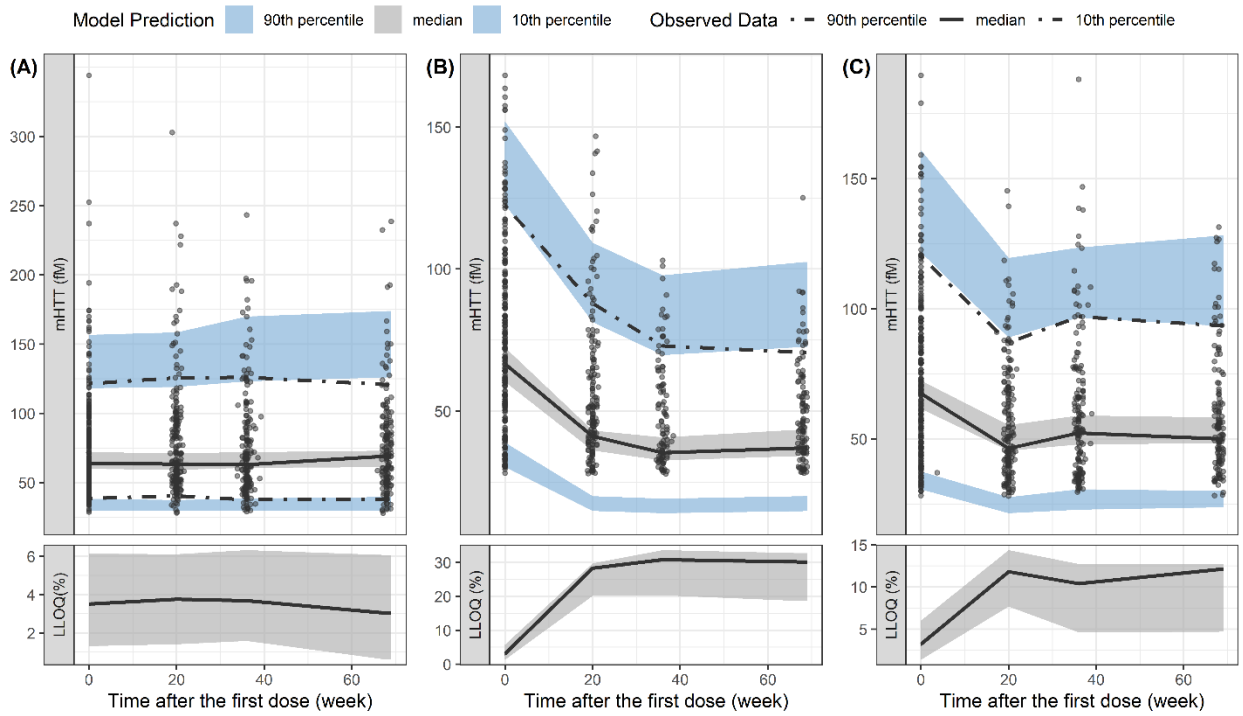

Figure 2

Prediction-corrected visual predictive check of the final PKPD model for (A) placebo, (B) Q8W, and (C) Q16W. Observed concentrations are shown as black circles, with median, 5th and 95th percentiles of the observed data as solid line, lower dashed line and upper dashed line, respectively. The gray shaded areas represent the 90% confidence interval (CI) of the medians, and the blue-shared areas represent the 90% CI of 5th and 95th percentiles predicted by the model. In the lower panels, the solid black line represents the observed fraction of samples below LLOQ, and the grey areas are the simulated 90% CI of the fraction below LLOQ.

LLOQ, lower limit of quantification; mHTT, mutant huntingtin protein in CSF; Q8W, every 8 weeks; Q16W, every 16 weeks. Q4W data is excluded from the plot due to a very limited amount of data.

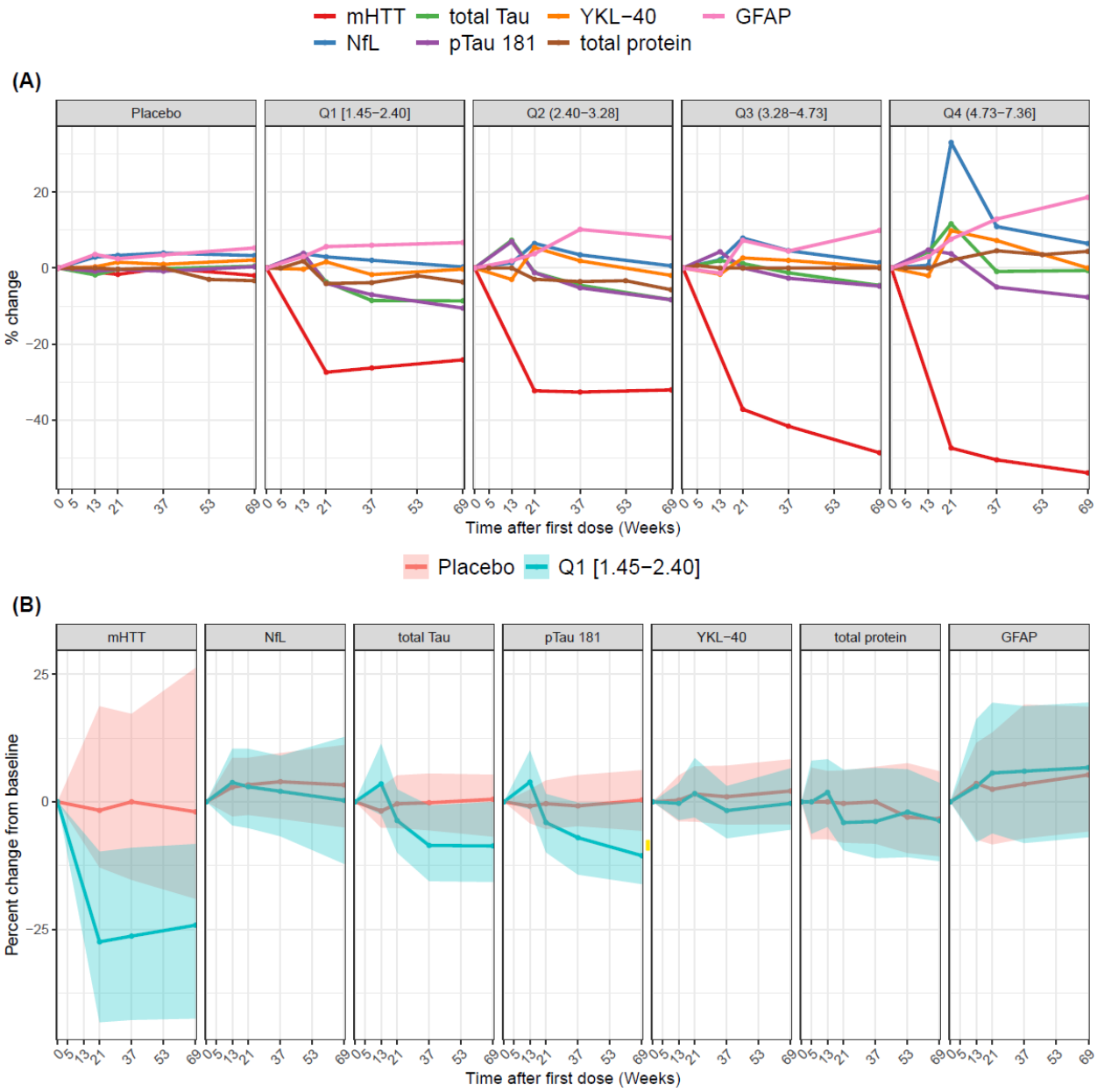

Figure 3

(A) Longitudinal percent change from baseline in CSF fluid biomarkers stratified by tominersen exposure ($C_{av,CSF,ss}$) quartile groups. (B) Longitudinal percent change from baseline in CSF fluid biomarkers colored by placebo or tominersen lowest exposure group. The lines are median, and shaded areas indicate the interquartile range (25^{th} to 75^{th} percentile).

mHTT, mutant huntingtin protein in CSF; NfL, neurofilament light chain in CSF; pTau 181, phosphorylated Tau 181; YKL-40, chitinase-3-like protein 1 in CSF; GFAP, glial fibrillary acidic protein.

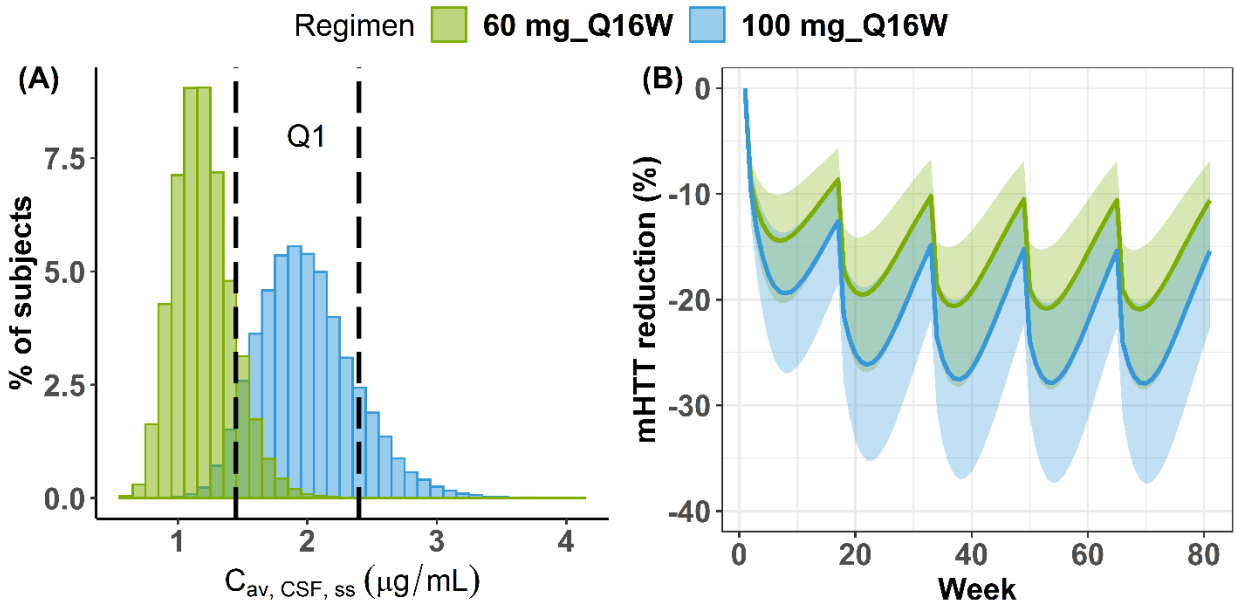

Figure 4

(A) Distribution of simulated $C_{av,CSF,SS}$ for 60 and 100 mg Q16W. The dotted lines indicate the range of the lowest exposure group (Q1) in GEN-HD1. (B) Simulated time-concentration profile of CSF mHTT reduction at 60 and 100 mg Q16W. The solid lines represent the median of the simulated data, and the corresponding shaded areas represent the 50% prediction interval of the simulated data.

CSF, cerebrospinal fluid; $C_{av,CSF,SS}$, average tominersen CSF concentration at steady state; GEN-HD1, GENERATION HD1; mHTT, mutant huntingtin protein in CSF; Q16W, every 16 weeks.