Assays to Evaluate an Allele-selective Approach for Lowering Mutant Huntingtin in Clinical Trials

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SUMMARY

- We are advancing WVE-003, an investigational allele-selective mHTT-lowering oligonucleotide for the treatment of Huntington's disease (HD), in the clinical trial SELECT-HD (NCT05032196). Due to the significance of wtHTT function for the health of the CNS and the potential for mHTT to disrupt wtHTT function, selectively lowering mHTT while preserving wtHTT protein expression and function may offer advantages over non-selective HTT-lowering approaches for the treatment of HD.
- Herein, we delineate our biomarker strategy to support the allele-selective mechanism of action for WVE-003.
- mHTT protein is present in CSF of patients with HD, where its levels have been associated with clinical signs of disease progression, such as lower cognitive function and more severe motor dysfunction.[1-3] Mutant huntingtin can be quantified in the CSF (mHTT assay), and this assay has been used by academia and industry to understand the natural history of mHTT and its response to HTT-lowering treatments.[1-9]
- Wild-type huntingtin is also detected in CSF, and we have developed an assay to quantify wild-type huntingtin (wtHTT assay).[10]
- In PRECISION-HD1 and PRECISION-HD2, clinical trials designed to evaluate first-generation allele-selective molecules, both assays performed consistently within the acceptance parameters and intended context of use. Results from the wtHTT assay suggest mHTT accounts for 30-40% of HTT protein in CSF of early manifest HD patients.
- These data reinforce confidence in our ability to evaluate the allele-selective mechanism of action of WVE-003 in the SELECT-HD trial.

INTRODUCTION

- The causative mutation in HD, called mutant HTT (*mHTT*), has pleiotropic effects on wtHTT protein function.[11]
- wtHTT is a scaffold protein that interacts with numerous protein partners, influencing various cellular processes, including vesicular and organelle trafficking, to support the health and function of neurons (**Figure 1**).
- The mutation in HTT can lead to a loss of wtHTT function, altered wtHTT function, and a dominant-negative effect on wtHTT function.
- Selectively lowering mHTT while preserving wtHTT protein expression and function may offer advantages over non-selective HTT-lowering approaches for the treatment of HD.

Figure 1. Rationale for allele-selective mHTT lowering approach

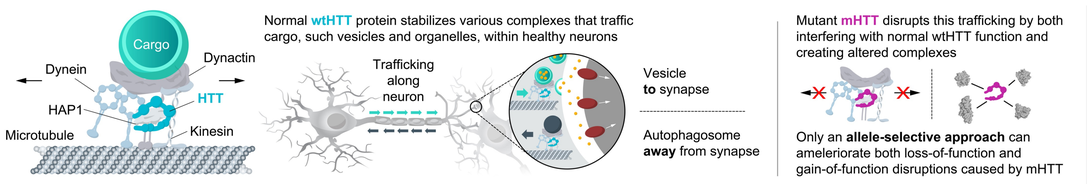
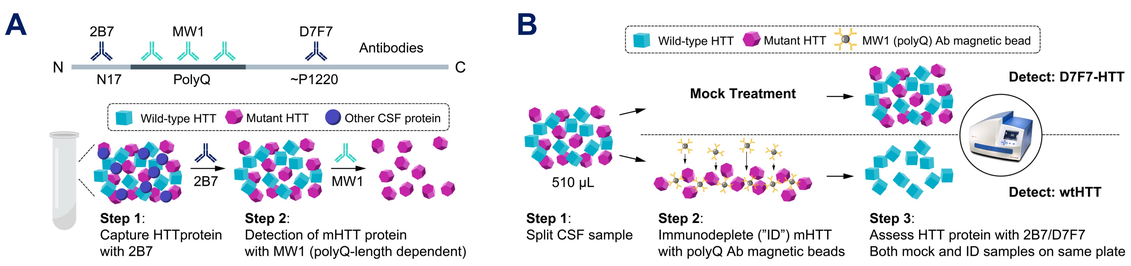


Figure 2. Overview of mHTT and wtHTT assays

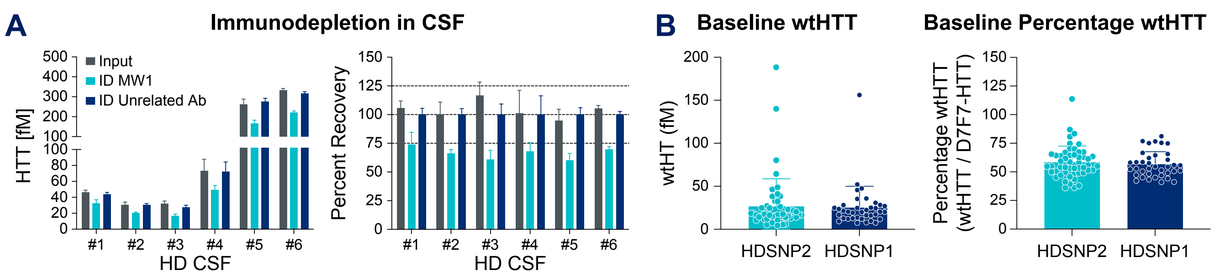


- To support the allele-selective mechanism of action of WVE-003, we use mHTT and wtHTT assays to quantify changes in these proteins in the CSF.
- These assays employ readily accessible antibodies (**Figure 2A**).
- The mHTT assay quantifies relative mHTT protein in the CSF (**Figure 2A**); this assay has been used by academia and industry to understand the natural history of mHTT and its response to HTT-lowering treatments.[1-9]
- Wild-type huntingtin is also detected in the CSF.[12] We developed a wtHTT assay to quantify relative wtHTT protein in the CSF (**Figure 2B**).[10]

The mHTT assay employs an N-terminal capture antibody, 2B7, conjugated to magnetic microparticles ("MP-2B7") and the polyQ-specific MW1 antibody coupled to Alexa-Fluor ("MW1-AF"). Recombinant HTT-Q46 protein (residues 1-548) is reconstituted in artificial CSF as calibrators and quality controls, at the levels noted. In brief, samples are incubated with MP-2B7 to enrich for HTT protein and thereafter incubated with MW1-AF. After washing, the complex is eluted for detection on the SMCxPro platform. The wtHTT assay similarly employs MP-2B7 for the capture step; however, the CSF sample is split and an additional immunodepletion step (or mock) is performed using MW1 antibody conjugated to magnetic particles ("MP-MW1"). Each split is assessed through the same detector antibody, D7F7 (epitope corresponding to residues around Pro1220 of human huntingtin protein), conjugated to a fluorescent label, detected on SMC Erenna Singulex. Recombinant HTT-Q48 protein (full length) and HTT-Q23 protein (full-length) is reconstituted in artificial CSF as calibrators and quality controls, at the levels noted.

RESULTS

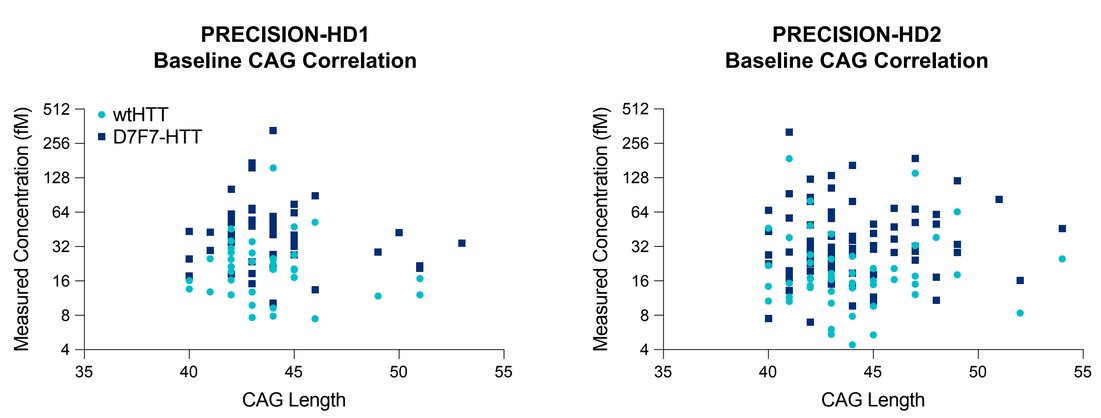
Figure 3. wtHTT protein accounts for 60%-70% HTT in CSF from patients with early onset HD



(**A**) wtHTT method qualification data for six individual HD CSF samples highlight similar post-immunodepletion recovery of HTT protein. Percent recovery calculated relative to ID Unrelated Ab control. An unrelated antibody was conjugated to magnetic particles as a negative control, compared with ID MW1 1:300, to evaluate specificity of the immunodepletion step. (**B**) Baseline wtHTT data from PRECISION-HD1 and PRECISION-HD2 cohorts are shown, totaling 37 and 51 subjects, respectively. The percentage wtHTT is the ratio of wtHTT to D7F7-HTT measured, multiplied by 100.

- When applied to early manifest patients with HD, immunodepletion of mHTT consistently yields a 30-40% reduction in HTT levels, indicating that mHTT accounts for 30%-40% of D7F7-HTT protein in the CSF of these patients (**Figure 3A**). These findings are consistent with prior reports[13] and with the notion that CSF mHTT levels increase with disease progression.[1-3]
- Mean wtHTT protein levels detected in CSF samples were approximately 25 fM in both PRECISION-HD1 and PRECISION-HD2 cohorts at baseline. These protein levels on average account for 50-60% of HTT protein measured by the D7F7-HTT assay (**Figure 3B**).

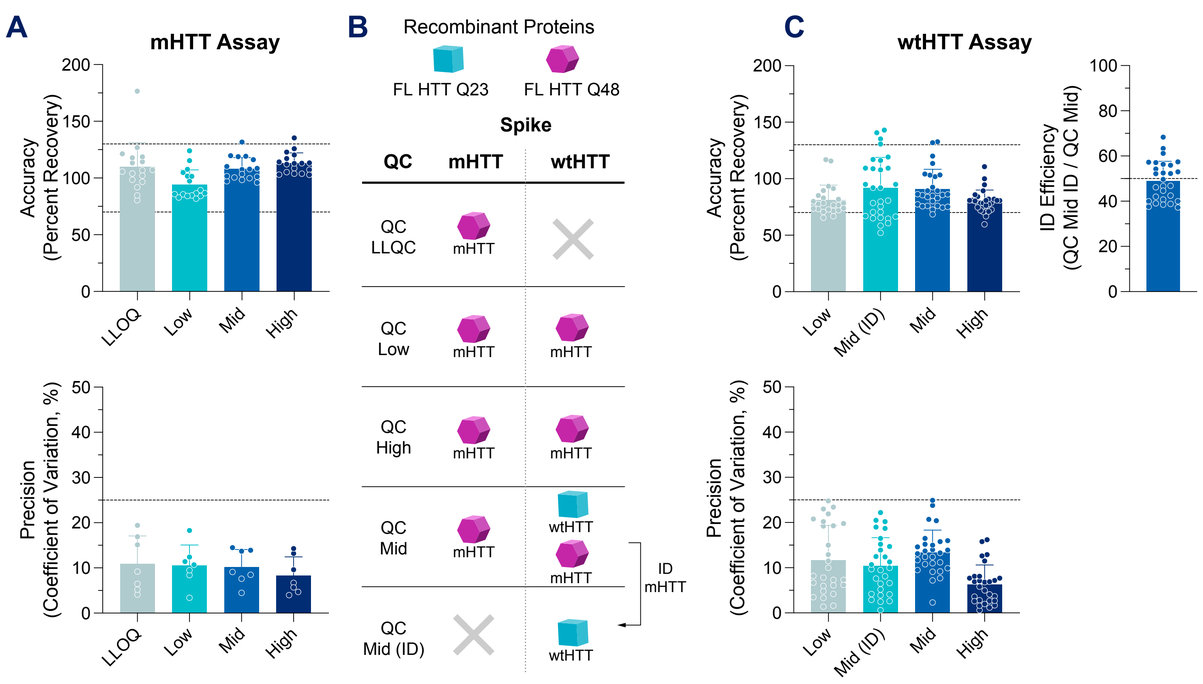
Figure 4. Application of D7F7-HTT and wtHTT assays to PRECISION-HD1 and PRECISION-HD2 CSF samples shows no correlation between CAG-repeat length and protein concentration



Scatter plots depict measured concentrations of either wtHTT or D7F7-HTT at baseline versus CAG length. No statistically significant correlations were found when comparing the measured concentration with CAG length.

- CSF wtHTT protein levels detected in baseline samples from the PRECISION-HD studies are not correlated with the length of the CAG repeats (**Figure 4**), supporting the notion that the assay specifically measures wtHTT in CSF (not dependent on polyglutamine repeat).

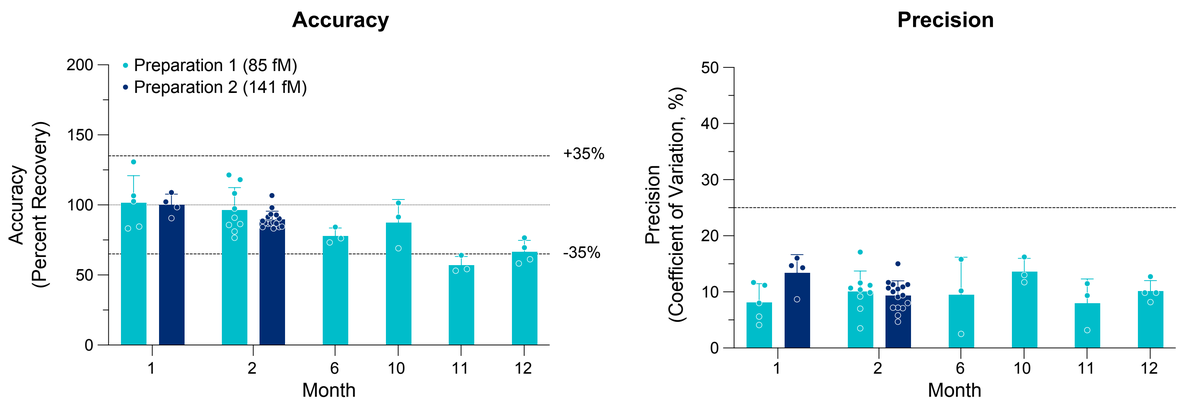
Figure 5. Both assays performed consistently within acceptance criteria parameters and intended context of use



Bar charts depict mean values for each level, overlaid with individual data for each triplicate assessment dervied from a plate run. (**A**) For the mHTT assay*, QCs are evaluated at the lower limit of quantification (LLOQ, 23.9 fM), low (89.6 fM), mid (1290 fM), and high (2670 fM) levels. (**B**) Recombinant HTT protein is truncated (1-548 Q46) for mtHTT assay. FL: full-length; HTT: huntingtin, ID; immunodeplete. (**C**) For the wtHTT assay, QCs are evaluated at the low (30 fM), mid (ID, 50 fM; Input 100 fM), and high (200 fM) levels. ID efficiency depicts the mean percent ratio of measured QC Mid (ID) to QC Mid, displaying individual values calculated for each plate. *mHTT assay performed with a separate vendor.

- Both assays performed consistently within acceptance criteria parameters and intended context of use. Representative data for both assays are shown, totaling 17 and 27 runs for the mHTT and wtHTT assays, respectively (**Figure 5A,C**).
- Quality control preparation scheme for mHTT and wtHTT assays is shown. For the mHTT assay, a truncated (1-548) recombinant huntingtin protein with a 46 polyglutamine repeat is spiked into artificial CSF. For the wtHTT assay, full-length recombinant HTT proteins with either a 23 or 48 polyglutamine repeat are spiked into artificial CSF. The QC Mid level is an equimolar spike of HTT-Q23 and HTT-Q48 and the QC Mid ID is the QC Mid subjected to immunodepletion with MW1-MPs (**Figure 5B**).
- The mean HTT concentration measured for the QC Mid level (after immunodepletion) is 49% of the input QC Mid level (intra-batch run), demonstrating reproducible and efficient immunodepletion (**Figure 5C**).

Figure 6. Wild-type HTT protein is detected in non-HD human CSF



Each data point represents the mean accuracy or precision of a triplicate well quantification of QC aliquots, evaluated at batch qualification (month 1) and later time points. QC batch qualifications are performed over 3-5 independent runs. Two independent batch preparations (pools of non-HD human CSF from ≥ 20 individuals) are shown.

- Measurement of HTT protein from pooled human non-HD CSF is reproducible and within 25% bias, on average, for up to 10 months.
- In healthy individuals, CSF wtHTT protein is detected at low femtomolar levels, consistent with prior studies.[6]

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