Preclinical Data in Support of WVE-003, an Investigational Antisense Oligonucleotide Designed to Selectively Lower Mutant HTT

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SUMMARY

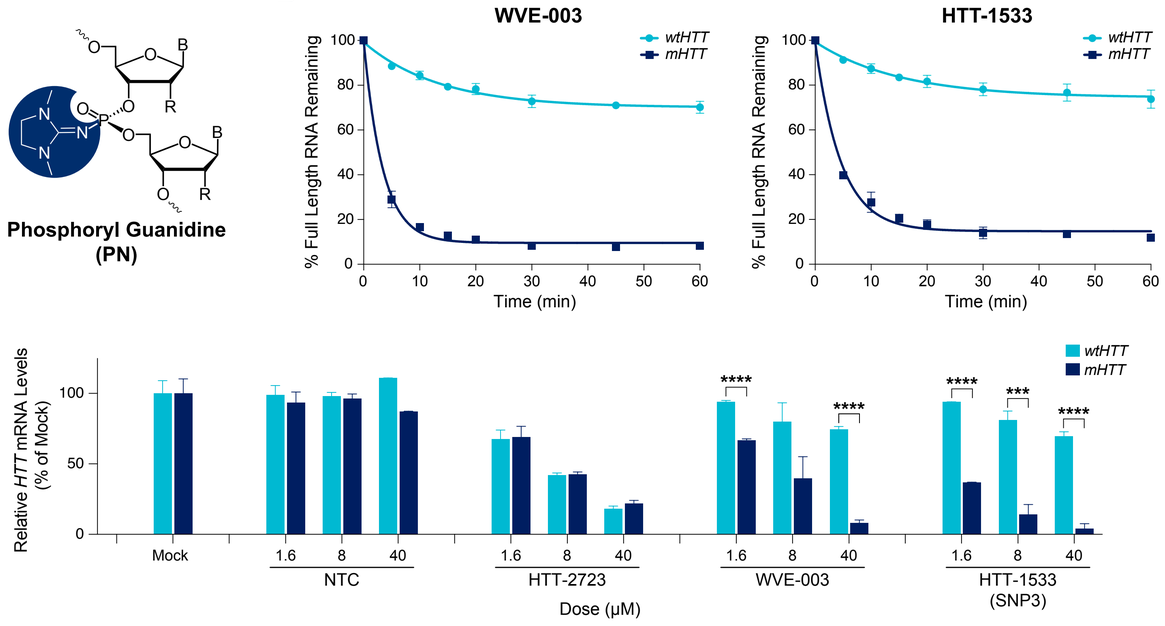
- We are evaluating WVE-003, an investigational allele-selective mHTT-lowering oligonucleotide for the treatment of Huntingtin's disease (HD), in a clinical trial called SELECT-HD (NCT05032196). Due to the significance of wtHTT function for the health of the CNS and the potential for mHTT to disrupt wtHTT function,[1,2] 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 highlight some of the preclinical data that supports the allele-selective mechanism of action of WVE-003.[3] HTT-2723 is a pan-silencing oligonucleotide designed based on tominersen.[4]
- WVE-003 and HTT-1533 are stereopure phosphorothioate (PS) and phosphoryl guanidine (PN)-containing oligonucleotides that target a variant of a single nucleotide polymorphism called SNP3.
- We demonstrate that SNP3-targeting molecules are potent, durable, and selective for mHTT *in vitro* in iPSC-derived neurons and *in vivo* in the Hu97/18 mouse model.
- HTT-1533 outperforms first-generation antisense oligonucleotides designed to target SNP1 or SNP2 *in vitro*.
- In the mice, HTT-1533 displays potency equivalent to HTT-2723, with respect to mHTT lowering. By contrast, HTT-1533 showed improved durability while sparing wtHTT.
- These and other preclinical findings support the allele-selective mechanism of action of WVE-003.

INTRODUCTION

- Incorporation of PN (phosphoryl guanidine) backbone modifications has been shown to improve the potency, durability, and distribution of silencing oligonucleotides in CNS in preclinical studies.[5] WVE-003 incorporates PN chemistry (**Figure 1A**).
- WVE-003 and HTT-1533, antisense oligonucleotides targeting SNP3, have similar allele-selective activity in biochemical RNase H assays (**Figure 1B**), with both promoting the preferential degradation of *mHTT* RNA.
- In iPSC patient-derived neurons heterozygous for SNP3, WVE-003 and HTT-1533 selectively lower *mHTT* transcript expression and preserve *wtHTT* expression, whereas HTT-2723 lowers both (**Figure 1C**).

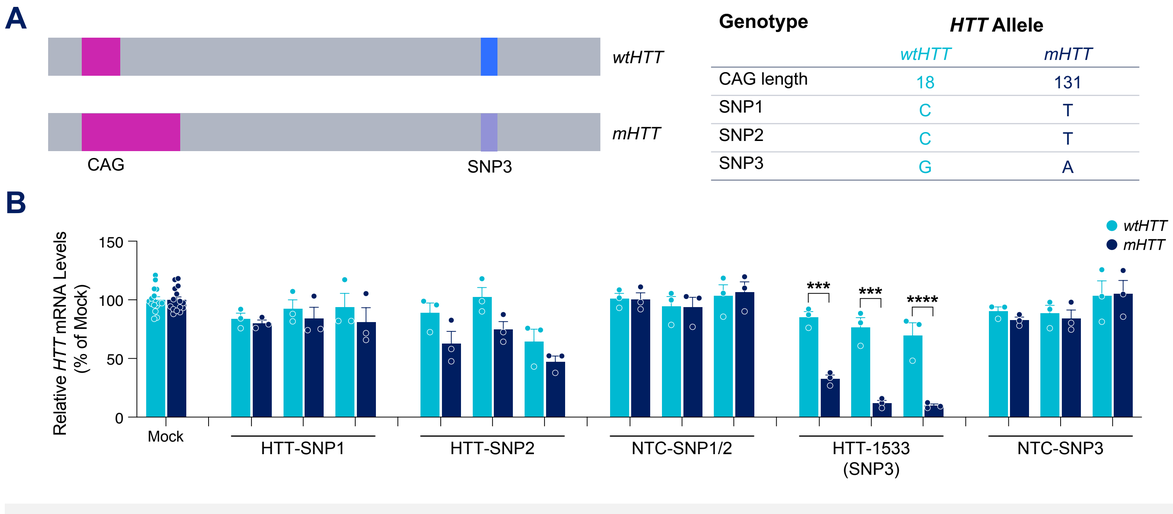
RESULTS

Figure 1. SNP3-targeting molecules are allele-selective *in vitro*



(**B**) RNase H experiments performed with synthetic RNA substrates corresponding to *wtHTT* and *mHTT* transcripts. The percentage of full-length RNA remaining in the presence of antisense oligonucleotide and RNase H was quantified at the indicated time points, n=3. (**C**) Patient-derived motor neurons (heterozygous SNP3) were treated with increasing concentrations of the indicated oligonucleotide (1.6-40 μM). Remaining percentage of *wtHTT* (cyan) and *mHTT* (navy) relative to mock-treated samples is shown, n=2. Stats: White-adjusted 3-way ANOVA with 2-tailed post-hoc tests comparing *mHTT* and *wtHTT* expression. *** p≤0.001, **** p≤0.0001.

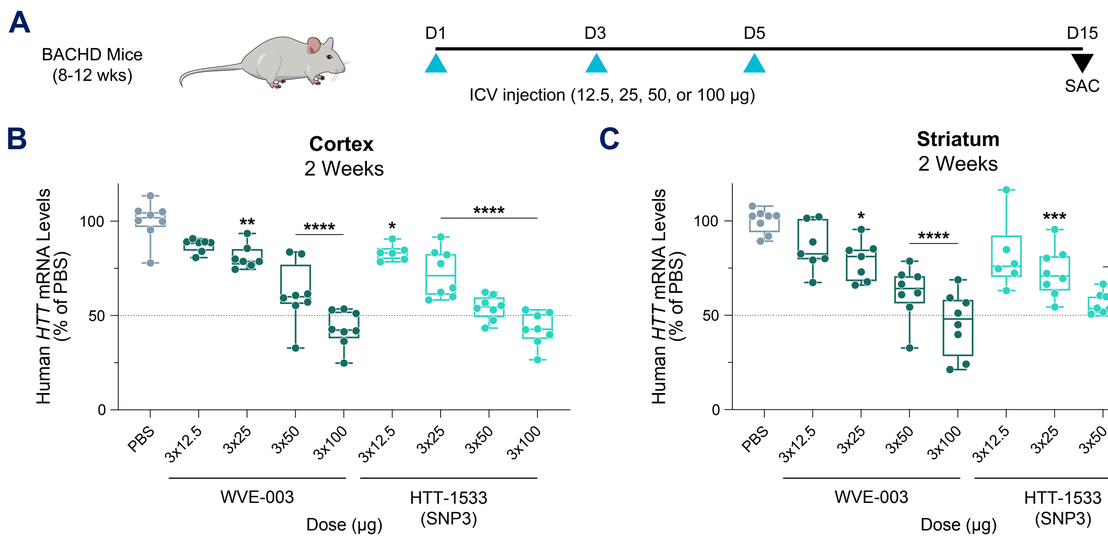
Figure 2. SNP3-targeting molecule outperforms SNP1- and SNP2-targeting molecules in heterozygous iPSC-derived neurons



(**B**) Patient iPSC-derived neurons heterozygous for SNP1, SNP2, and SNP3 were treated with the indicated oligonucleotides or non-targeting control (NTC) at increasing concentrations (1.6, 8.0, or 40 μM) for 7 days. Percentage of *mHTT* (cyan) and *wtHTT* (navy) transcript expression relative to mock treated control cells is shown, n=3. Stats: 3-way ANOVA with 2-tailed post-hoc comparisons of *mHTT* to *wtHTT* *** p<0.001; **** p<0.0001.

- We previously evaluated allele-selective oligonucleotides targeting SNP1 or SNP2 in PRECISION-HD clinical trials (NCT03225833, NCT03225846). These molecules were discontinued in 2021, because they did not consistently lower CSF mHTT protein levels in patients with HD.[6]
- We identified a commercially available iPSC-line that was heterozygous for SNP1, SNP2, and SNP3, and that has the desired phasing between the SNP variants and the CAG-repeat region[7] (**Figure 2A**).
- In neurons derived from this iPSC line, HTT-1533 was more potent and selective than SNP1 or SNP2-targeting oligonucleotides (**Figure 2B**).

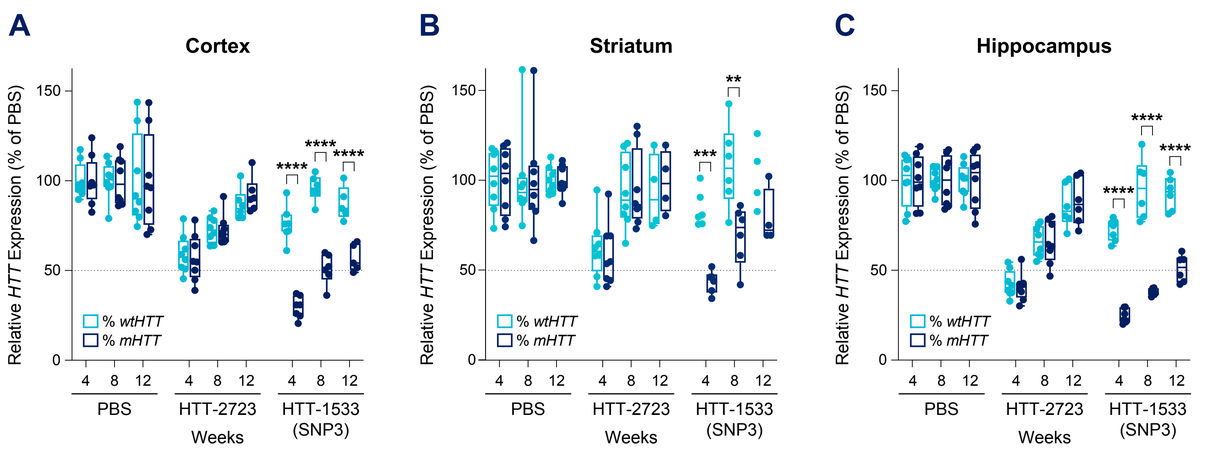
Figure 3. SNP3-targeting oligonucleotides dose-dependently decrease *mHTT* expression in CNS of BACHD mice



(**B**,**C**) BACHD mice were treated with increasing concentrations of the indicated SNP3-targeting oligonucleotide or PBS by intracerebroventricular injection. The relative fold change of *mHTT* transcript expression is shown as a percentage of PBS in the cortex (**B**) and striatum (**C**), n=7-8. Stats: One-way ANOVA followed by two-tailed post-hoc comparisons to PBS *p≤0.05, ** p≤0.01, *** p≤0.001, ****p≤0.0001.

- To assess activity *in vivo*, we evaluated HTT-1533 and HTT-2723 in BACHD mouse model,[8] which contains SNP3 on some copies of the human *mHTT* transgene.[3]
- In the CNS of BACHD mice, WVE-003 and HTT-1533 dose-dependently and significantly lowered *mHTT* transcript expression in cortex (**Figure 3B**) and striatum (**Figure 3C**).
- WVE-003 and HTT-1533 exhibited similar activity profiles in BACHD mice.

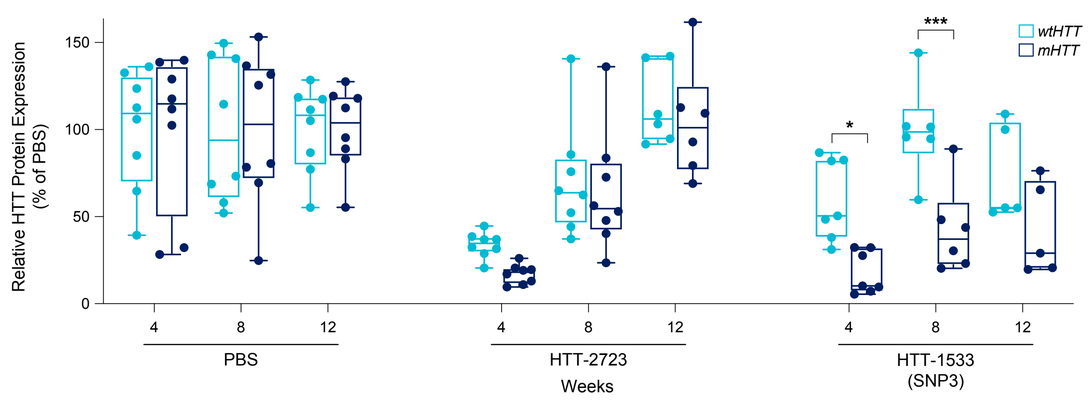
Figure 4. HTT-1533 lowers *mHTT* and preserves *wtHTT* transcript expression in CNS of Hu97/18 mice



Hu97/18 were treated with the indicated oligonucleotide (3 x 100 μg) or PBS by intracerebroventricular injection. The relative fold change of *mHTT* or *wtHTT* transcript expression is shown as a percentage of PBS in the cortex (**A**), striatum (**B**), or hippocampus (**C**), n=6-8. Stats: White-adjusted 3-way ANOVA with post-hoc test comparing *mHTT* to *wtHTT* expression ** p≤0.01, *** p≤0.001, ****p≤0.0001.

- To assess activity *in vivo*, we evaluated HTT-1533 and HTT-2723 in the fully humanized Hu97/18 mouse model, which contains SNP3 on the human *mHTT* allele, expresses human *wtHTT*, and lacks expression of mouse *Htt*.[9]
- In the CNS of Hu97/18 mice, HTT-1533 selectively lowered *mHTT* transcript expression in cortex (**Figure 4A**), striatum (**Figure 4B**), and hippocampus (**Figure 4C**) for at least 12-weeks post-dose, while relatively preserving expression of *wtHTT*.
- By contrast, HTT-2723 lowered both *mHTT* and *wtHTT* transcript expression throughout the CNS.

Figure 5. HTT-1533 lowers mHTT and preserves wtHTT protein expression in cortex of Hu97/18 mice



Mice were treated as in Figure 4. mHTT and wtHTT protein in cortex from treated mice were quantified by western blot. Relative mHTT and wtHTT protein expression are shown, n=6-8. Stats: 3-way ANOVA with 2-tailed post-hoc comparison of mHTT to wtHTT at each time point, p ≤ 0.05, *** p ≤ 0.001.

- We evaluated the impact of HTT-1533 and HTT-2723 on HTT proteins in the Hu97/18 mouse model.
- In the cortex, HTT-1533 selectively lowered mHTT protein (**Figure 5**), while relatively preserving expression of wtHTT protein for up to 8 weeks post-injection.
- By contrast, HTT-2723 lowered both mHTT and wtHTT protein comparably throughout the CNS at all timepoints evaluated.

References: 1. Saudou & Humbert. 2016. *Neuron*, doi: 10.1016/j.neuron.2016.02.003; 2. Bragg et al., 2024. *bioRxiv*, doi: 10.1101/2024.01.11.575238; 3. Iwamoto et al., submitted, Preclinical evaluation of stereopure antisense oligonucleotides for allele-selective lowering of mutant *HTT;* 4. Tabrizi et al., 2019. *N Engl J Med*, doi: 10.1056/NEJMoa1900907; 5. Kandasamy et al., 2022. *Nucleic Acids Res*, doi: 10.1093/nar/gkac037; 6. https://ir.wavelifesciences.com/news-releases/news-release-details/wave-life-sciences provides-update-phase-1b2a-precision-hd; 7. Svrzikapa et al., 2020. *Mol Ther Methods Clin Dev*, doi: 10.1016/j.omtm.2020.09.003; 8. Gray et al., 2008. *J Neurosci*, doi: 10.1523/JNEUROSCI.0857-08.2008; 9. Southwell et al., 2013. *Hum Mol Genet*, doi: 10.1093/hmg/dds397. **Acknowledgments:** The authors are grateful to UCLA for the BACHD line developed by Dr. Xiangdong W. Yang; to Dr. Amber Southwell for the Hu97/18 mouse model; and to Maria Frank-Kamenetsky and Jeff Brown for early contributions to this project. Amy Donner (Wave Life Sciences) and Eric Smith provided editorial and graphical support, respectively, for this poster.