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WVE-N531 with PN Backbone Modification Significantly Enhances Drug Concentrations in Heart, Diaphragm, and Skeletal Muscles in Non-human Primates

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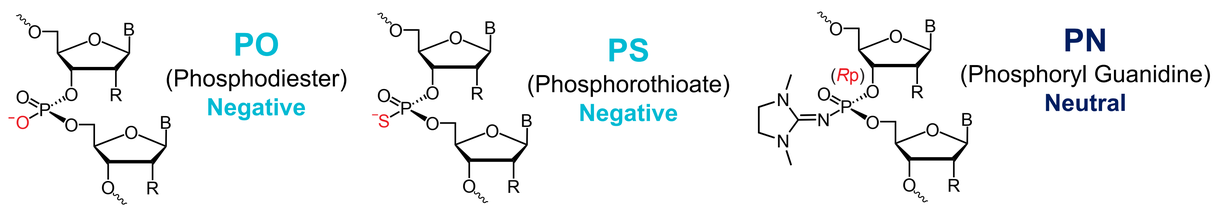
SUMMARY

- WVE-N531 is an investigational stereopure antisense oligonucleotide with novel phosphoryl guanidine (PN) chemistry currently being developed as a potential therapy for patients with Duchenne Muscular Dystrophy (DMD) amenable to exon 53 skipping.
  - Wave's PN chemistry had a substantial impact on muscle exposure, exon skipping, dystrophin restoration, survival, and both respiratory and skeletal muscle function in preclinical studies.[1]
- In Part A of a Phase 1b/2 open-label study involving three ambulatory boys, WVE-N531 reached a mean concentration of 42 µg/g in skeletal muscle tissue and yielded 53% mean exon skipping (RT-PCR) following three 10 mg/kg doses administered every other week.
  - Mean dystrophin production was 0.27% (BLQ) of normal as measured by western blot.
  - The Phase 2 portion of the study (FORWARD-53 [Part B]) is designed to assess dystrophin protein restoration over an extended period and in a larger population.
- To investigate the potential of WVE-N531 in addressing cardiorespiratory complications experienced by patients with DMD, we evaluated the distribution of WVE-N531 in the cardiac, diaphragm, and skeletal muscle tissues of cynomolgus monkeys.
- WVE-N531 achieved high mean concentrations in skeletal muscle in cynomolgus monkeys, with even higher general exposure observed in the heart and diaphragm.
  - The mean tissue concentrations of WVE-N531 were 57.2 µg/g in heart, 10.8 µg/g in diaphragm, and 2.17 µg/g in skeletal muscle (tibialis anterior) after administration of four biweekly (every other week) doses at 15 mg/kg.
  - The 15 mg/kg dose level in this monkey study was approximately equivalent to 10 mg/kg in patients based on area under the plasma drug concentration-time curve (AUC) values.
- These data illustrate WVE-N531's excellent tissue uptake and distribution in both skeletal and non-skeletal muscles and support the advancement of WVE-N531 in clinical testing (FORWARD-53; NCT04906460).

INTRODUCTION

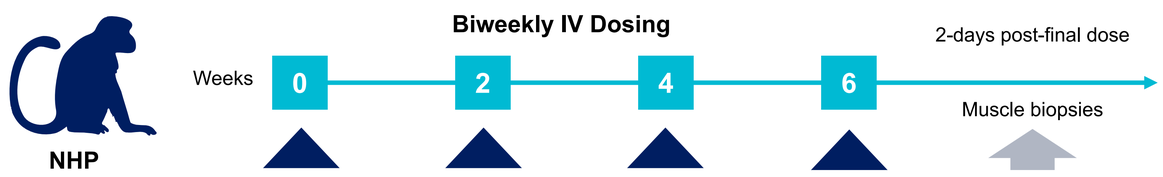
- Duchenne muscular dystrophy (DMD) is a X-linked neuromuscular disorder in which mutations in the dystrophin gene result in absent or defective dystrophin protein.[2]
- Dystrophin protein plays a key structural role in muscle fiber function,3 and lack of dystrophin protein results in severe, progressive muscle atrophy, eventual loss of ambulation, respiratory insufficiency, cardiomyopathy, and premature death.[2,4]
- Despite advances, there remains a substantial unmet need for treatments that reach cardiac and respiratory muscles, where restoration of dystrophin may be essential to improve respiratory and cardiac function and extend survival.[5-7]
- WVE-N531 is an investigational stereopure exon 53 skipping antisense oligonucleotide that contains PN chemistry (**Figure 1**).
- WVE-N531 was designed to address the limitations of first-generation exon-skipping oligonucleotides that had modest muscle delivery, including in the heart and diaphragm.[8]
- Here we present preclinical data on the concentrations of WVE-N531 in skeletal and non-skeletal muscles of non-human primates (NHP) following 6 weeks of biweekly intravenous infusion.

Figure 1. WVE-N531 is an investigational stereopure antisense oligonucleotide with novel PN backbone chemistry



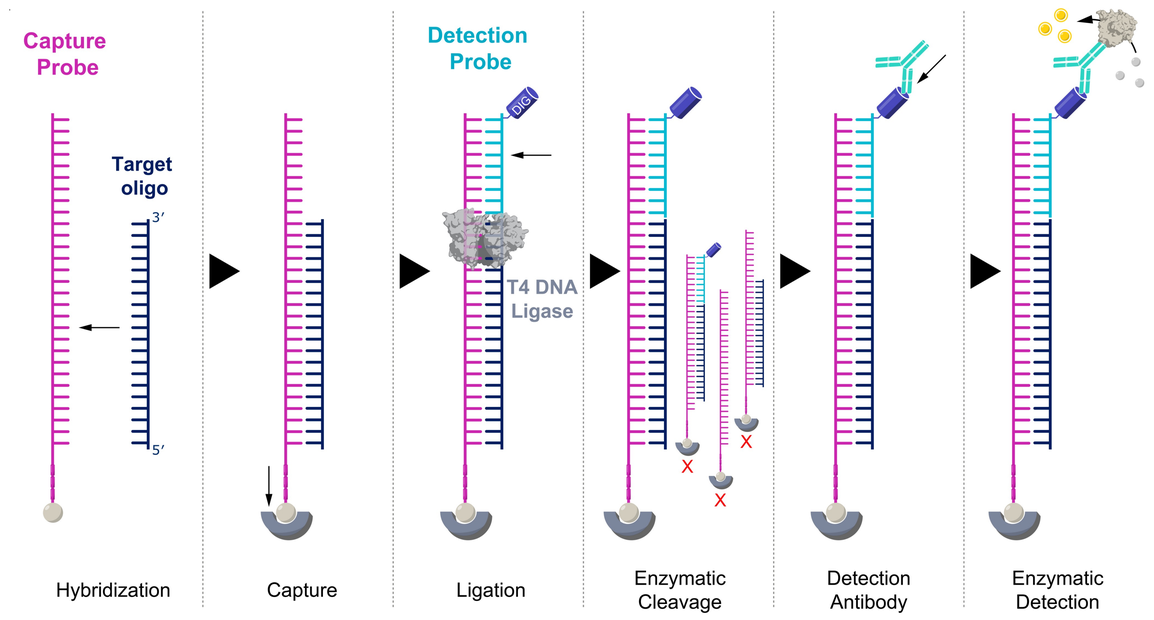
STUDY OBJECTIVE, DESIGN AND METHODS

Figure 2. Study design: Repeat dosing in NHP



Data include muscle concentrations of WVE-N531.

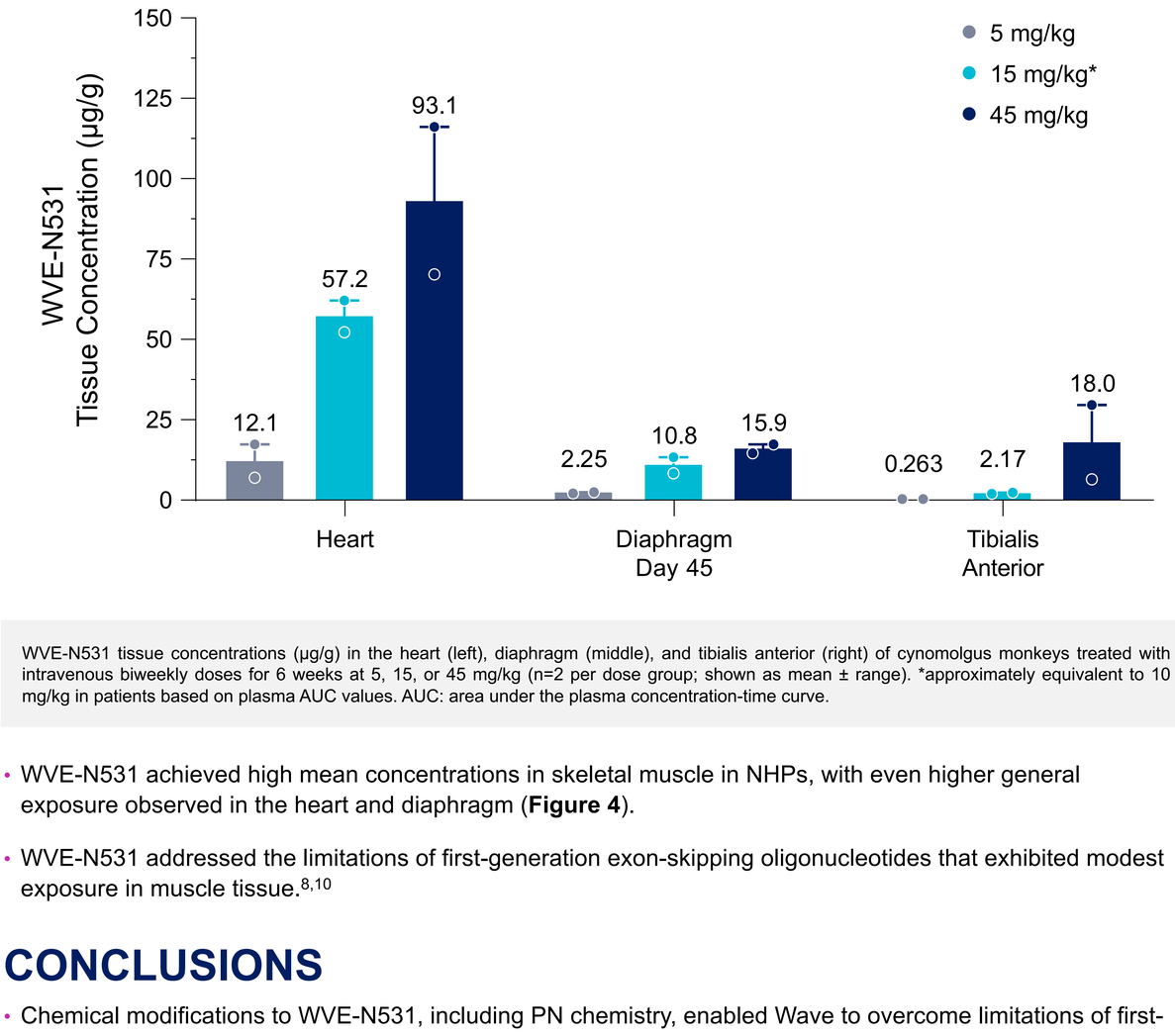
Figure 3. Hybridization-ligation ELISA (HL-ELISA)



In HL-ELISA, WVE-N531 is hybridized to the WVE-N531 capture probe (Hybridization and Capture), which is biotinylated at the 3'-end. After binding, the WVE-N531 detection probe, which is conjugated to digoxigenin on the 5'-end, is hybridized to WVE-N531 by DNA ligase (Ligation). After detection binding, S1 nuclease is added to digest the unhybridized probes (Enzymatic cleavage). An anti-digoxigenin antibody conjugated to alkaline phosphatase is used to detect the hybridized analyte by binding to digoxigenin (Detection Antibody). AttoPhos® is used as a substrate for fluorometric readout. Fluorescence intensity is determined using a fluorescent plate reader (Enzymatic detection).

- The objective of this study was to evaluate the concentrations of WVE-N531 in skeletal and non-skeletal muscle tissue of cynomolgus monkeys.
- WVE-N531 was administered every other week via intravenous infusion to cynomolgus monkeys for 6 weeks at 5, 15, or 45 mg/kg (n=2 per dose group) (**Figure 2**).
- Concentrations of WVE-N531 in cardiac, diaphragm, and skeletal muscle were measured at two days post-last dose using hybridization-ligation enzyme-linked immunosorbent assay (HL-ELISA) (**Figure 3**).[9]

Figure 4. WVE-N531 cardiac, diaphragm, and skeletal muscle tissue concentrations in monkeys



WVE-N531 tissue concentrations (µg/g) in the heart (left), diaphragm (middle), and tibialis anterior (right) of cynomolgus monkeys treated with intravenous biweekly doses for 6 weeks at 5, 15, or 45 mg/kg (n=2 per dose group; shown as mean ± range). *approximately equivalent to 10 mg/kg in patients based on plasma AUC values. AUC: area under the plasma concentration-time curve.

- WVE-N531 achieved high mean concentrations in skeletal muscle in NHPs, with even higher general exposure observed in the heart and diaphragm (**Figure 4**).
- WVE-N531 addressed the limitations of first-generation exon-skipping oligonucleotides that exhibited modest exposure in muscle tissue.[8,10]

CONCLUSIONS

- Chemical modifications to WVE-N531, including PN chemistry, enabled Wave to overcome limitations of first-generation exon-skipping oligonucleotides by significantly enhancing concentrations in heart, diaphragm, and skeletal muscles in NHPs.
- These results are consistent with observations from other preclinical models using a PN-containing surrogate[1] and indicate that it may be possible to attain comparably high levels of WVE-N531 in human skeletal, heart, and diaphragm muscle tissue.
  - Notably, a Phase 1b/2 open-label study has already demonstrated in three boys that WVE-N531 achieved a mean concentration of 42 µg/g in human skeletal muscle tissue after three biweekly 10 mg/kg doses.
  - The 15 mg/kg dose level in the monkey study was approximately equivalent to 10 mg/kg in patients based on plasma AUC values.
- WVE-N531's high tissue uptake and distribution in both skeletal and non-skeletal muscles support the advancement of WVE-N531 in clinical testing (FORWARD-53; NCT04906460).

References: 1. Kandasamy P, et al. *Nucleic Acids Res*. 2022 Jun 10;50(10):5401-5423. doi: 10.1093/nar/gkac037; 2. Mah JK. *Neuropsychiatr Dis Treat*. 2016 Jul 22;12:1795-807; 3. Blake DJ, Weir A, Newey SE, and Davies KE. *Physiol Rev*. 2002 Apr;82(2):291-329; 4. Boland BJ, Silbert PL, Groover RV, Wollan PC, and Silverstein MD. *Pediatr Neurol*. 1996 Jan;14(1):7-12; 5. Fortunato F, Farnè M, and Ferlini A. *Neuromuscul Disord*. 2021 Oct;31(10):1013-20; 6. Meyers TA and Townsend D. *Int J Mol Sci*. 2019 Aug;20(17):4098; 7. Sheehan DW, et al. *Pediatrics*. 2018 Oct;142(Supplement_2):S62-71; 8. Phan H, et al. Interim Analysis of the Phase 1 Open-Label Extension Study of Suvodirsen in Patients With Duchenne Muscular Dystrophy Amenable to Exon 51 Skipping. 2020 Mar. Presented at: MDA Clinical & Scientific Conference; 9. Haegele JA, Boyanapalli R, and Goyal J. *Nucleic Acid Ther*. 2022 Aug;32(4):350-9; 10. Verhaart IEC and Aartsma-Rus A. *Nat Rev Neurol*. 2019 Jul;15(7):373-386.
Acknowledgments: For development of this poster, the authors thank Amy Donner and Alexander Lin (Wave Life Sciences) for medical writing support and Eric Smith for graphics support. This work was funded by Wave Life Sciences. **Disclosures:** All authors are employees of Wave Life Sciences.