M168

First Clinical Evidence for Stem Cell Targeting in DMD: Results from Part A of a Phase 1b/2 Study of WVE-N531

WAVE LIFE SCIENCES

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SUMMARY

- WVE-N531 is an investigational stereopure antisense oligonucleotide with novel PN (phosphoryl guanidine) chemistry currently being developed as a potential therapy for patients with Duchenne Muscular Dystrophy (DMD) amenable to exon 53 skipping (**Figure 1**).
- In Part A (n=3) of a Phase 1b/2 clinical trial (NCT04906460), WVE-N531 yielded 53% mean exon skipping (RT-PCR) and reached a mean concentration of 42 µg/g in muscle tissue after three doses administered at 10 mg/kg every other week.
  - RNAscope (*in situ* hybridization) demonstrated that WVE-N531 reached the myocyte nucleus.
  - Mean dystrophin production was 0.27% (BLQ, western blot) of normal; extended dosing and follow up are needed to confirm increased production of dystrophin over time.
- WVE-N531 was generally safe and well-tolerated, with most adverse events being mild in intensity (Part A safety analysis, December 2022).
- Upon further analysis of the muscle biopsies, WVE-N531 demonstrated clear uptake in stem cells in all three patients as evaluated by a dual paired box protein 7 (PAX7, stem cell marker) immunohistochemistry and WVE-N531 RNAscope chromogenic assay.
- This finding represents the first clinical evidence of a potential therapeutic for DMD having the ability to access stem cells.
- Based on the encouraging results from Part A, 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.

INTRODUCTION

- DMD is a severe neuromuscular disorder caused by mutations in the gene encoding dystrophin, a protein essential for maintaining the structural integrity of myofibers.[1]
- In addition to the myofiber phenotype, DMD also affects early myogenic stem cells or satellite cells. Stem cells play a crucial role in DMD pathogenesis including muscle regeneration, and the absence of dystrophin in these cells results in reduced asymmetric cell division and myogenic commitment, as well as increased mitotic stress, abnormal cell division events, and cellular senescence (**Figure 2**).[2-5]
- Telomere shortening in stem cells has been suggested to be associated with disease severity and progression. DMD patients have telomeres that are between 25% and 40% shorter than those in healthy individuals (**Figure 2**).[4,5]
- Most therapeutic strategies target the dystrophic myofibers, and do not address the cell-autonomous defects of dystrophin-deficient stem cells. Accessing these elusive stem cells, which augment muscle regenerative capacity, remains a significant challenge.
- We evaluated the uptake of WVE-N531 by skeletal muscle stem cells using biopsies from three patients enrolled in Part A of a Phase 1b/2 study.

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

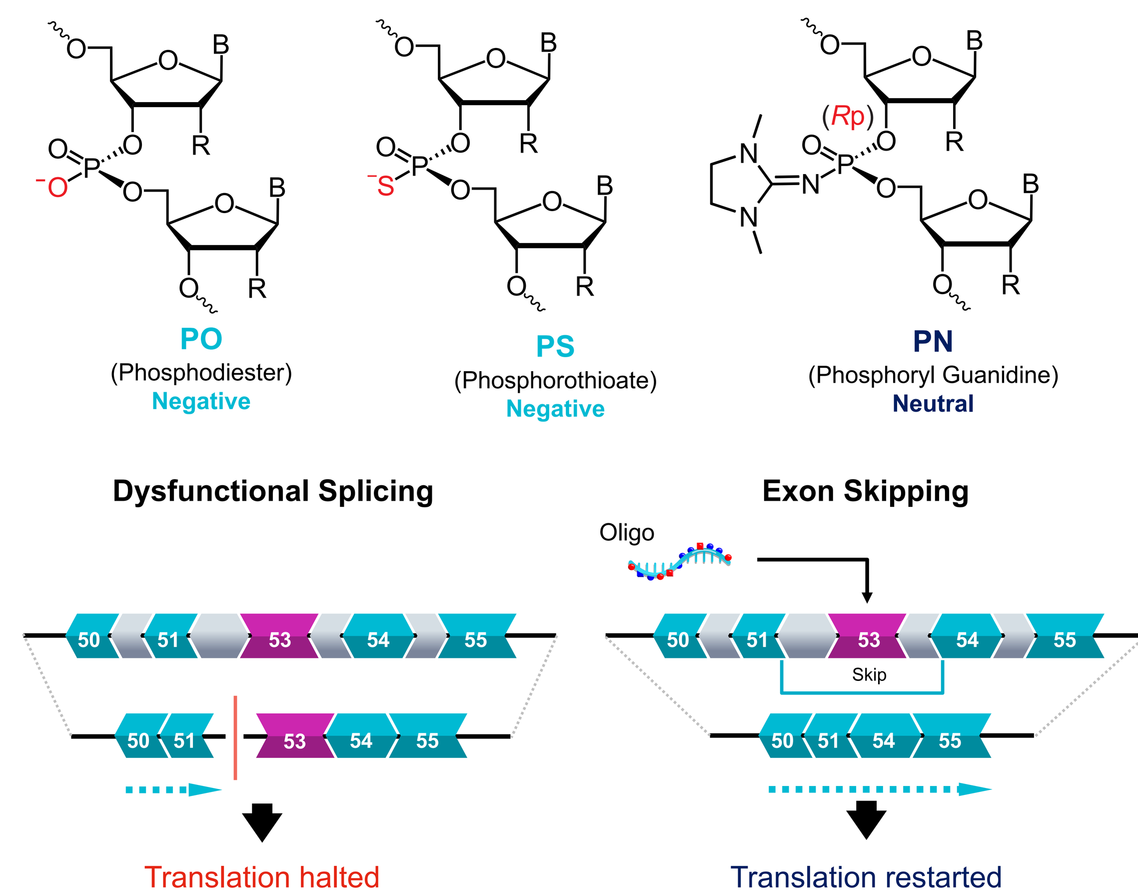
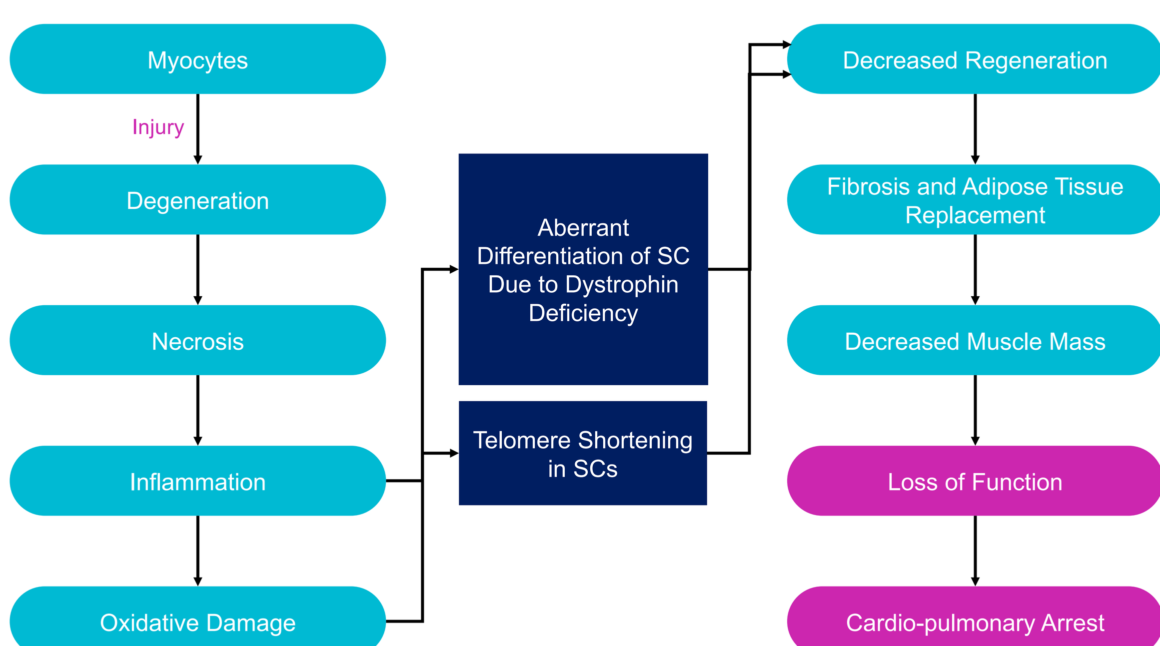
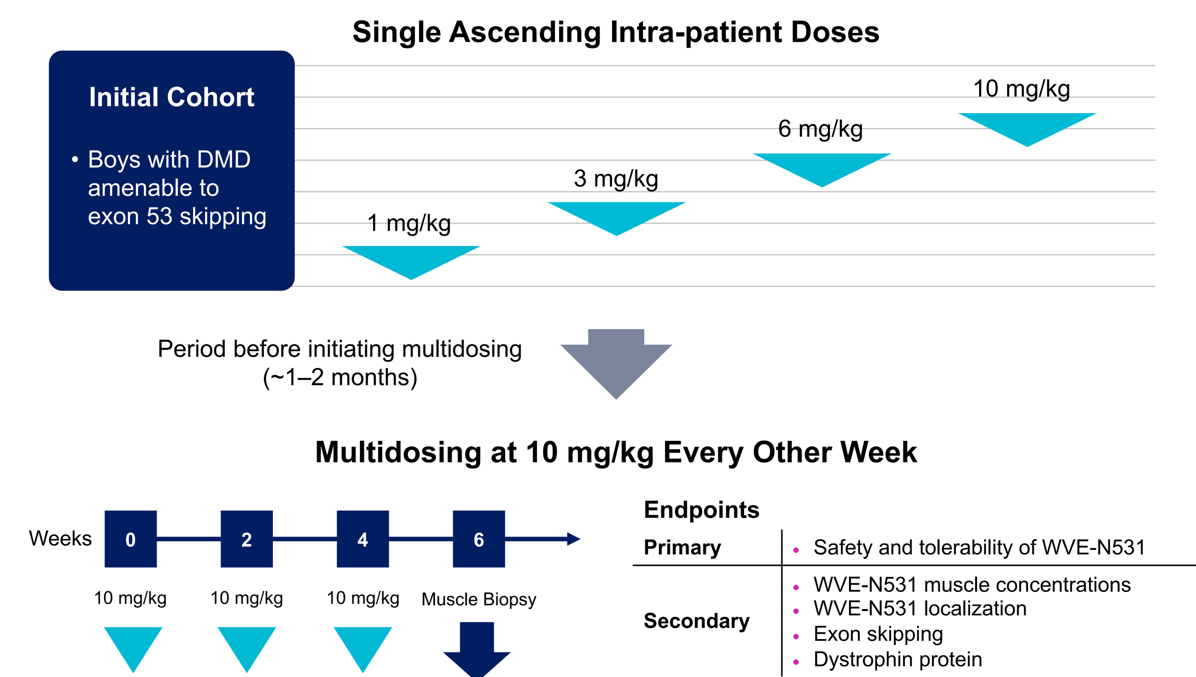


Figure 2. DMD Pathogenesis



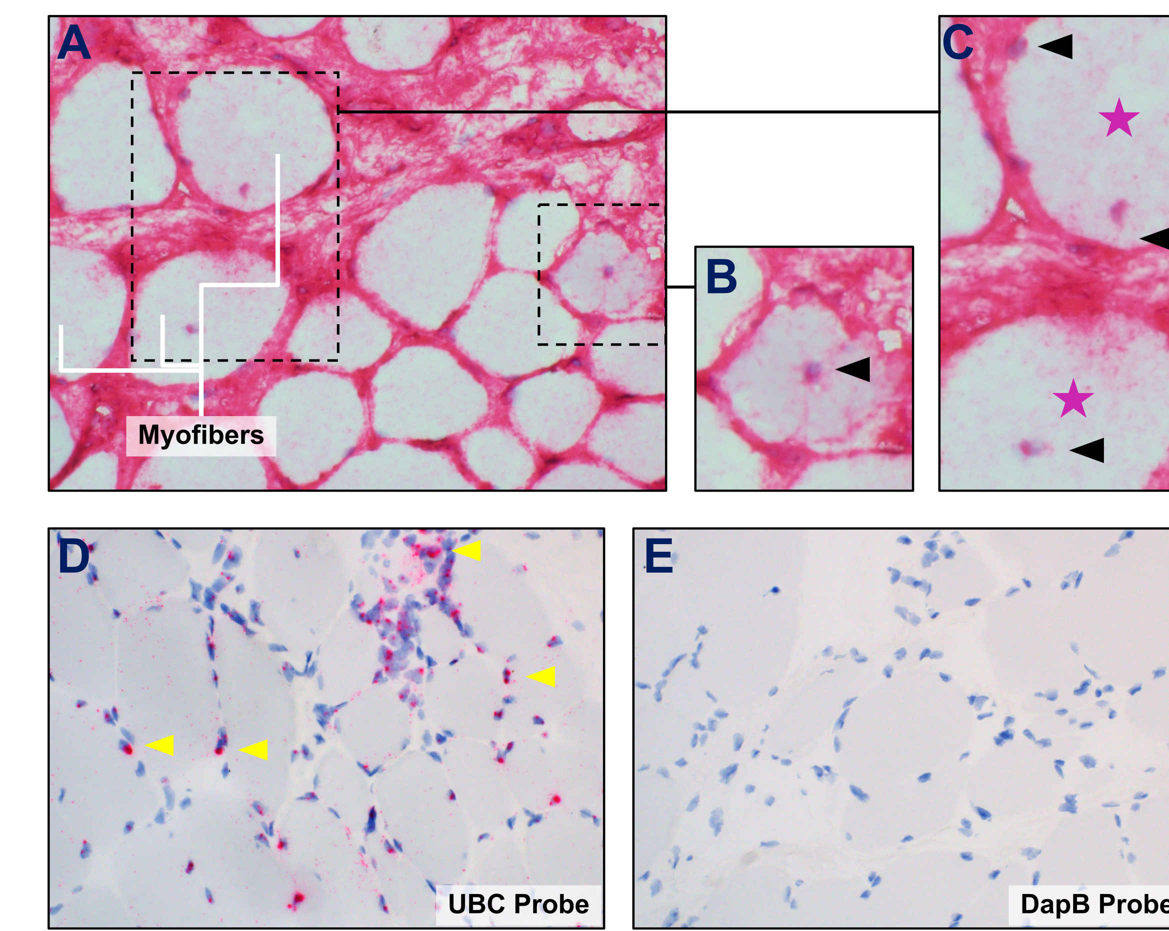
Adapted from Kottlors and Kirschner,[4] and Ribeiro , et al.[5] Abbreviation: SC, Stem Cells.

Figure 3. In multidose portion of Part A, patients received three biweekly 10 mg/kg doses



- The initial cohort in Part A of the study included three ambulatory boys who received single escalating doses of 1, 3, 6 and 10 mg/kg (administered ≥4 weeks apart). In the 6-week multidose portion of the study, the same boys received three doses of 10 mg/kg every other week (**Figure 3**).
- A muscle biopsy occurred 2 weeks after completion of multiple-dose treatment.

Figure 4. Intracellular WVE-N531 visualized in myofiber by *in situ* hybridization (RNAscope)

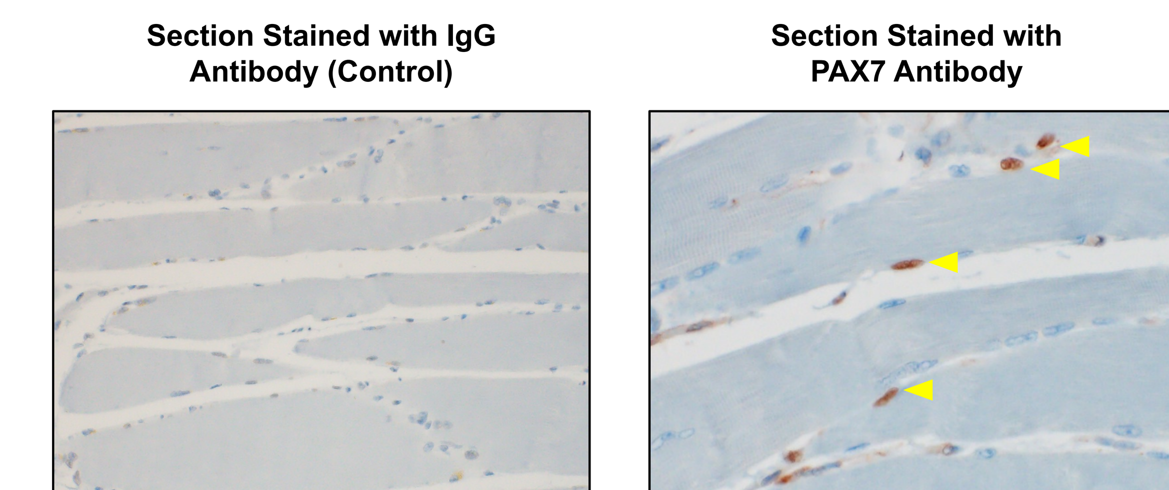


WVE-N531 (in red) in myofiber cytoplasm (stars) and nuclei (black arrowheads). (**A**) WVE-N531 probe (Magnification: 40x); (**B**,**C**) Zoomed in WVE-N531 probe; (**D**) Ubiquitin (UBC) – Positive control probe showing ubiquitin mRNA (yellow arrowheads); (**E**) DapB – Negative control probe. Magnification: 20x for control probes.

- At the 10 mg/kg dose level after the 6-week multidose portion of the study:
  - WVE-N531 reached high muscle concentrations (mean 42 µg/g), which was comparable to preclinical experiments in double knock out mice where a surrogate showed functional and survival benefits.
  - RNAscope (*in situ* hybridization) provided evidence that WVE-N531 reached the myofiber nucleus and enabled exon skipping (**Figure 4**).
  - Mean exon skipping was 53% (range, 48-62%) as measured by RT-PCR.
  - Mean dystrophin production was 0.27% (BLQ) of normal as measured by western blot. Extended dosing and follow up are needed to confirm increased production of dystrophin over time.

METHODS

Figure 5. Protocol optimization - PAX7 IHC on human skeletal muscle

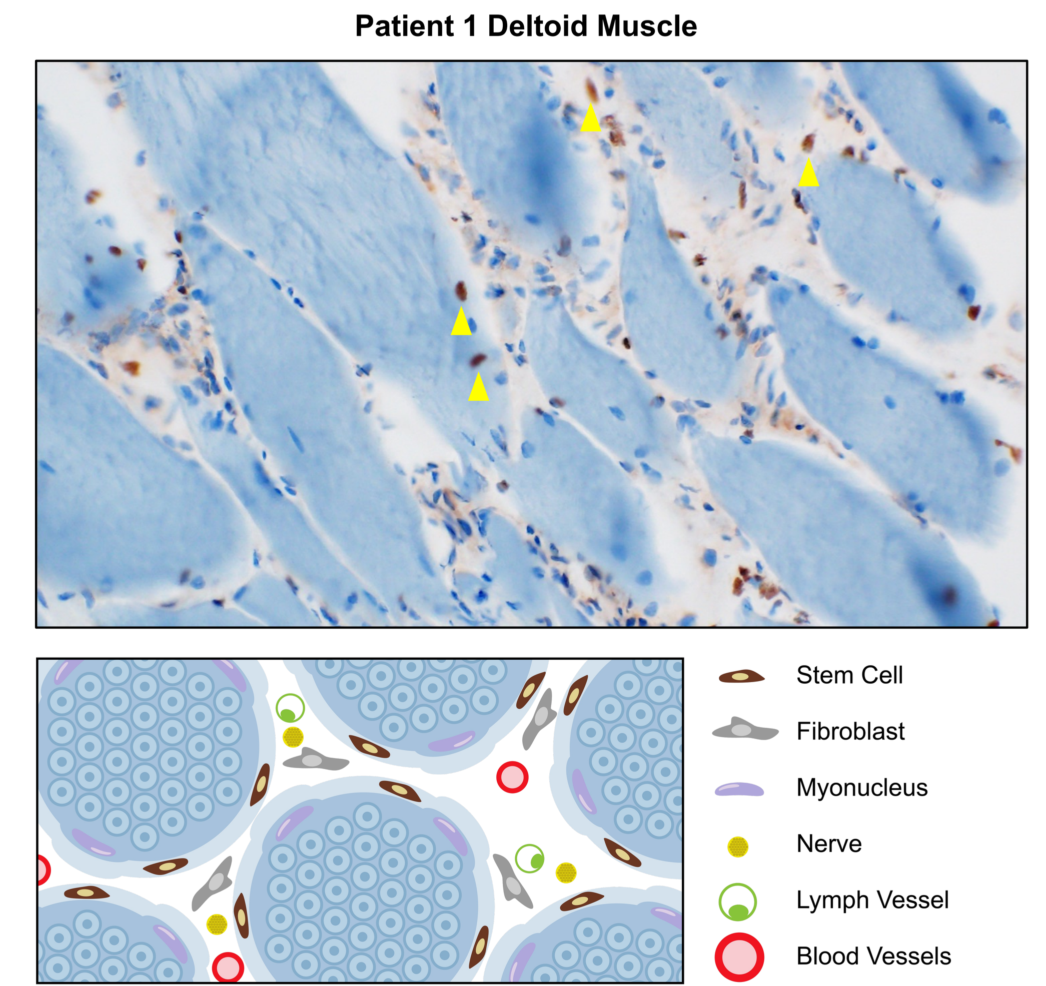


Original mag: 20x and 40x. Note PAX7 positive stem cells (arrows) confirming specificity. Background cytoplasmic signal in endothelial and smooth muscle cells. Abbreviation: IHC, immunohistochemistry.

- Paired box protein 7 (PAX7) is a transcription factor that plays a role in myogenesis by regulating the proliferation of precursor cells and is a validated biomarker for myocyte stem cells (**Figure 5**).
- PAX7 IHC protocol summary: 1:50 dilution used CC1 antigen retrieval (AR) without heat for 32-minute incubation.
- RNAScope + PAX7 IHC dual assay protocol summary: ACD WVE-N531 probe used at 1:50 dilution, 2 hr for 43°C on automated Vantana (Discovery Ultra). PAX7 IHC 1:40 dilution (without protease) with CC1 AR and heat for 60-minute incubation.

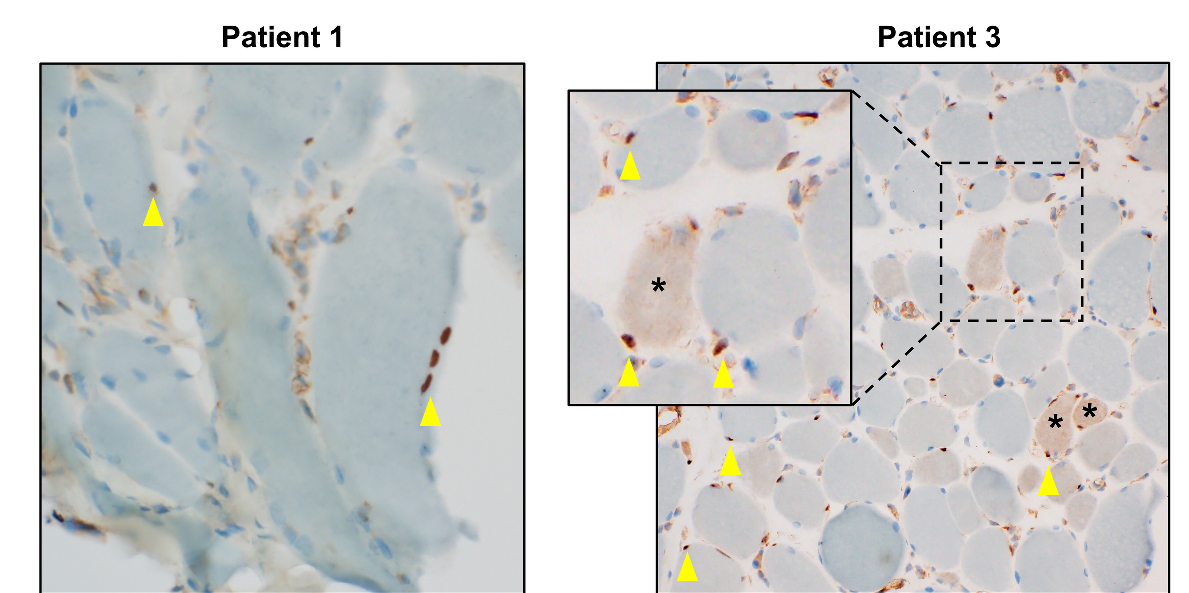
RESULTS

Figure 6. Observation of stem cells in patient samples



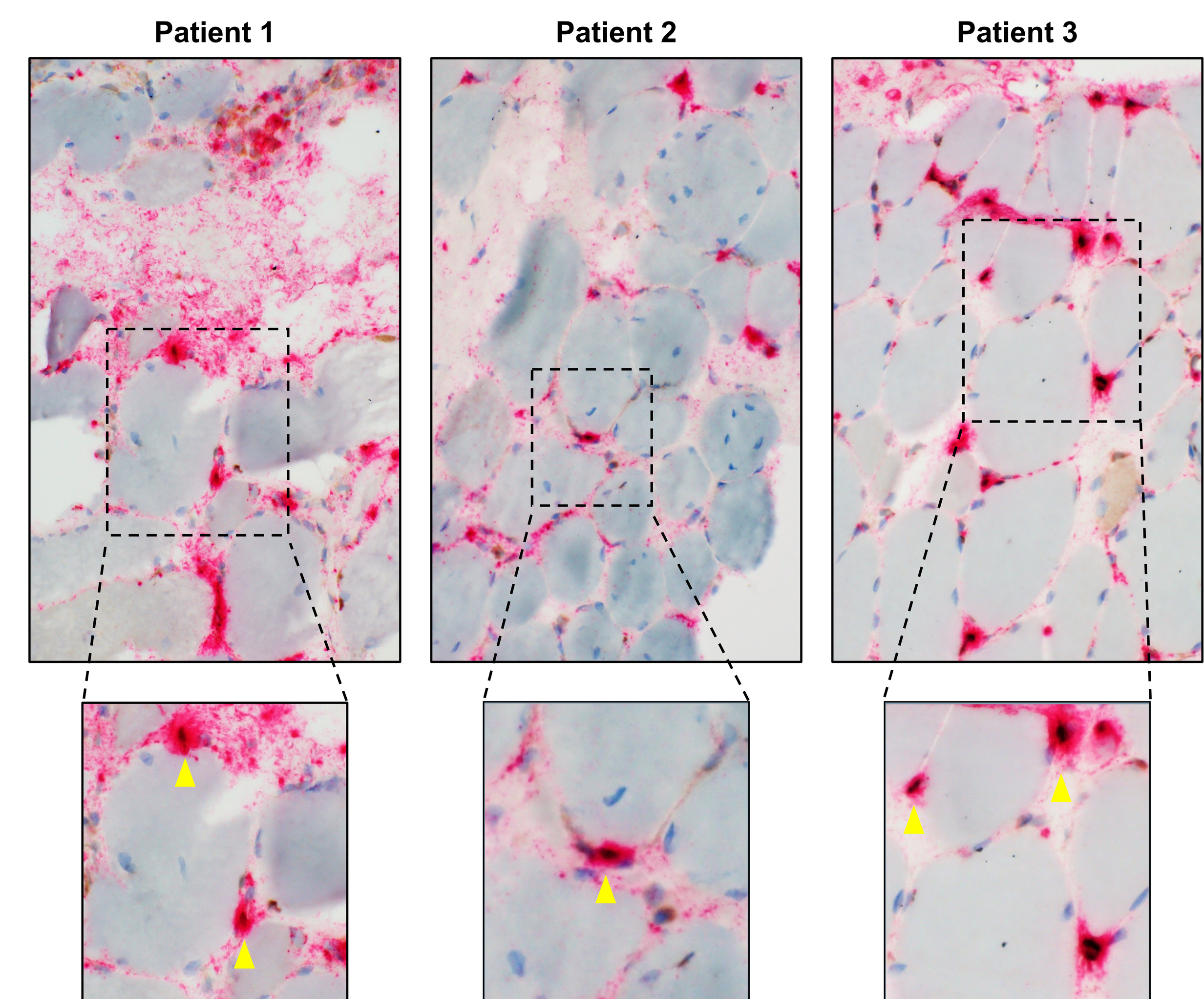
Note PAX7 positive stem cells (arrows). Background cytoplasmic signal in endothelial and smooth muscle cells.

Figure 7. Stem cell immunohistochemistry: Localization of stem cells in myofibers undergoing necrosis and repair



Original mag: 20x and 40x. Controls: IgG for PAX7 – Negative, Serial sections are not available. Note myocyte stem cells (yellow arrows) and necrotic myofibers (asterisks).

Figure 8. WVE-N531 uptake in myocyte stem cells



Controls: RNAScope Ubiquitin – Positive, & RNAScope DapB – Negative. Serial sections are not available.

- Stem cells were observed in patient samples and were mostly associated with myofibers undergoing necrosis and subsequent repair (**Figure 6**, **Figure 7**).
- WVE-N531 demonstrated clear uptake in stem cells in all three patients as evaluated by a dual PAX7 (stem cell marker) immunohistochemistry and WVE-N531 RNAscope chromogenic assay (**Figure 8**).
- Relatively increased numbers of stem cells were observed in samples from patient 3.
- There was variability in the number of PAX7 positive cells containing WVE-N531 among the three patients.
- Some background signal was observed with PAX7 IHC. Therefore, confirmation of these results using another method will be helpful.

References: 1. Blake DJ, et al. Physiol Rev. 2002 Apr;82(2):291-329; 2. Kodippili K and Rudnicki MA. *Frontiers in Physiology*. 2023 May;14:1180980; 3. Filippelli RL and Chang NC. *Cells Tissues Organs*. 2022 Dec;211(6):641-54; 4. Kottlors M and Kirschner J. *Cell and Tissue Research*. 2010 Jun;340:541-8; 5. Ribeiro Jr AF, et al. *Scientific Reports*. 2019 Aug;9(1):11842.

Acknowledgments: For development of this poster, the authors thank Alexander Lin (Wave Life Sciences) for medical writing support and Eric Smith for graphics support. This work was funded by Wave Life Sciences.