First clinical evidence for myocyte stem (satellite) cell targeting in DMD: Results from Part A of a Phase 1b/2 study of WVE-N531

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May 19, 2024

Presented at MENA 2024

Disclosures

Kuldeep Singh, Laurent Servais, Craig Campbell, Michael Tillinger, Xiao Shelley Hu, Andrew Hart, Joseph A. Haegele, Jeanette Rheinhardt, Anamitra Ghosh, Fangjun Liu, Chandra Vargeese, Anne-Marie Li-Kwai-Cheung, Padma Narayanan

- Kuldeep Singh is a full-time employee of Wave Life Sciences
- Laurent Servais has provided consultancy and lectures, and attended advisory boards for Sarepta, Dyne, Pfizer, Santhera, RegenxBio, Affinia, and Fibrogen
- Craig Campbell has served as site investigator for Acceleron, AMO, Biogen, Dyne, Fibrogen, Pfizer, Roche, PTC, Sarepta, Cytokinetics, Ultragenix, and Wave and has acted as a Data Safety Monitoring Board member for Catabasis, Edgewise, and Solid. Additionally, he has received investigator-initiated grants from Genzyme, PTC Therapeutics, and Biogen
- All other authors are employees of Wave Life Sciences

Overview

Phase 1b/2 Part A study design and results

WVE-N531 uptake in myogenic stem (satellite) cells

- Relevance of myogenic stem cell physiology and pathology in DMD
- Selection and protocol optimization of stem cell marker Paired Box Protein 7 (PAX-7) IHC and ISH
- Results and comments

Conclusions and next steps

Acknowledgements

WVE-N531: An investigational phosphoryl guanidine (PN)-containing ASO being developed for patients with DMD amenable to exon 53 skipping

Phase 1b/2 (Part A): First-in-human study results

- High exon skipping and muscle concentrations after three doses administered at 10 mg/kg every other week
- Similar exon skipping regardless of mutation
- PK analysis indicated 25-day half-life in plasma
- WVE-N531 appeared safe and well-tolerated

Patient	Tissue Source	Tissue concentration (ng/g)	% Exon skipping by RT-PCR	Dystrophin by western blot (% of normal)
1	Deltoid	85,500	61.5	0.24
2	Deltoid	33,500	49.8	0.23
3	Bicep	8,280	47.9	0.34

Mean muscle concentration: 42,400 ng/g

Mean exon skipping: 53%

Mean dystrophin: 0.27% of normal (BLQ)

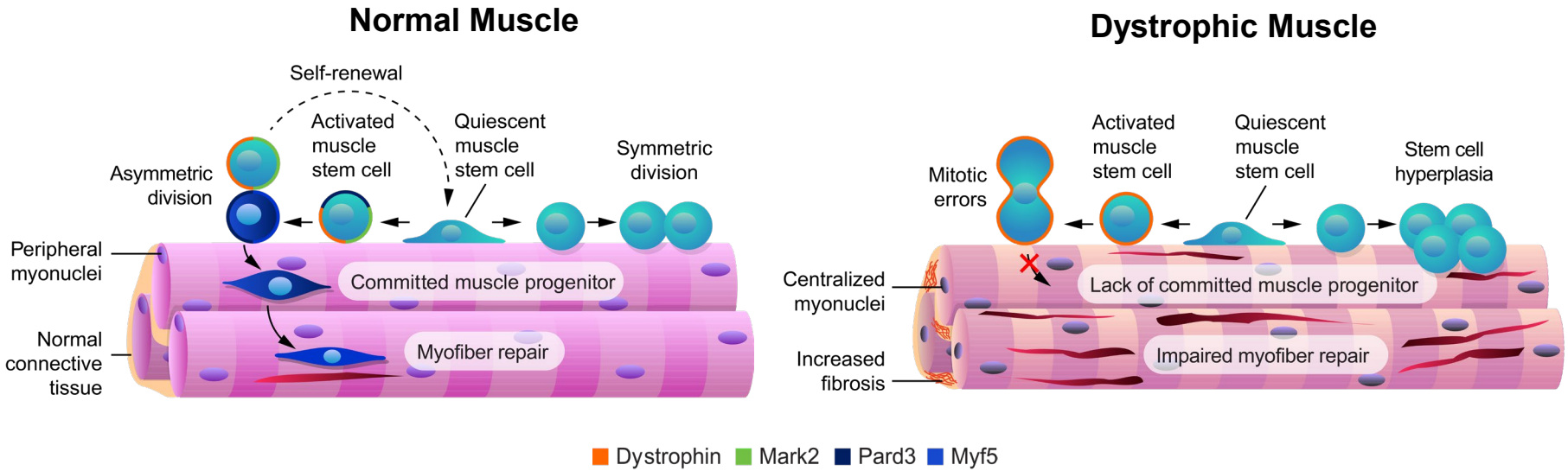
Biopsies collected ~2 weeks post-last dose (3 biweekly doses of 10 mg/kg)
Data cut-off: December 6, 2022

42,400 ng/g = 6,120 nM

BLQ: Below level of quantification (1%)

Why assess stem cell uptake?

- Stem cells play a major role in muscle regeneration
- Lack of dystrophin in stem cells results in stem cell dysfunction and impaired regeneration



- Stem cells use dystrophin, Mark2, and Pard3 to maintain polarity that is needed to undergo asymmetric cells division as it results in maintenance of stem cell pool while giving rise to myogenic progenitors.
- In DMD, loss of dystrophin in stem cells result in impaired cell polarity, reduced asymmetric cells divisions, and thus reduced regeneration.

Adapted from Keefe and Kardon, 2015

Experimental design: Myocyte stem cell uptake of WVE-N531

- The **objective** was to assess WVE-N531 uptake by skeletal muscle stem cells using biopsies from the 3 patients enrolled in Part A of the Phase 1b/2 study
- **Rationale:** Skeletal muscles of DMD patients show impaired differentiation and regeneration by stem cells due to lack of dystrophin[1]
- **Hypothesis:** WVE-N531 uptake by stem cells would restore stem cell differentiation and hence regeneration. For this part of analysis, the goal is to show WVE-N531 uptake in these cells
- **Stem cell marker:** Paired Box Protein 7 (PAX7)
- **Assay:** Dual PAX7 IHC and WVE-N531 RNAScope

[1] Kotters and Kischner 2010 and Ribeiro et al., 2019

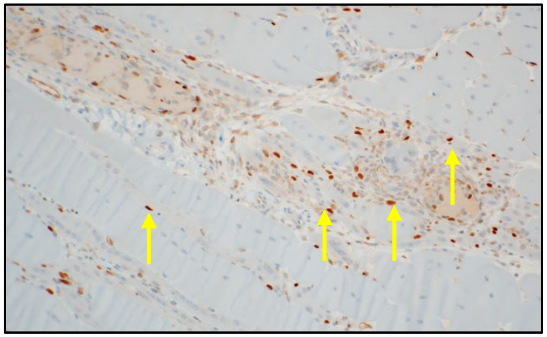
Protocol optimization for PAX7 IHC: muscle from *mdx* mouse and healthy human

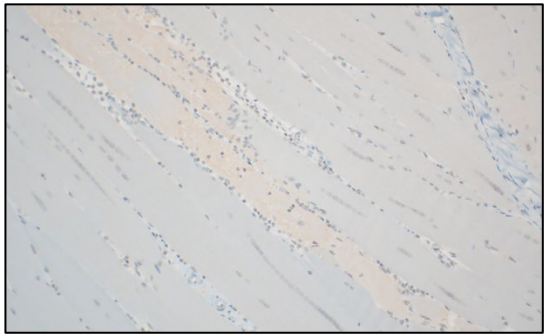
Anti PAX7 Antibody - NBP232894

Sections stained with PAX7 antibody

Sections stained with IgG antibody (control)

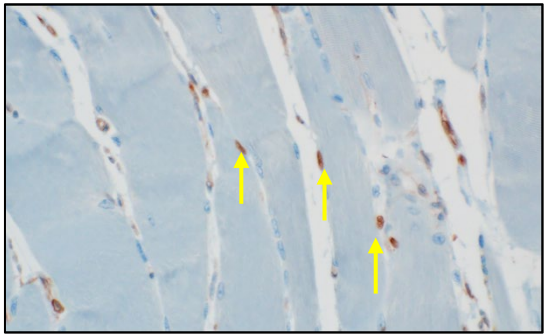
WVE-N531 Treated Mouse

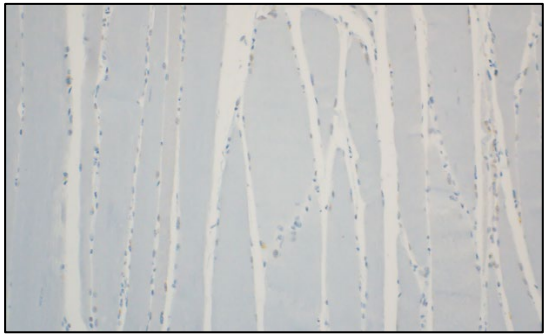




WVE-N531 Treated Mouse

Healthy human (WVE-N531 untreated)





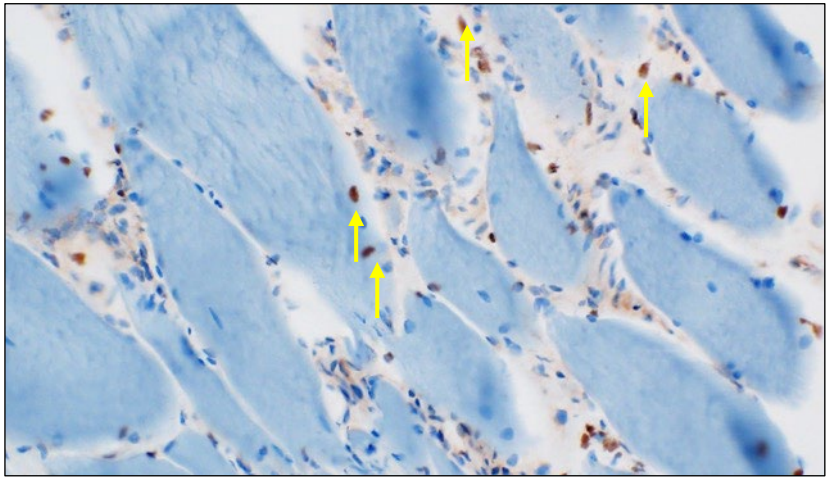
Healthy human (WVE-N531 untreated)

Note multiple PAX7 positive stem cells confirming specificity. Yellow arrows point to some of the positive nuclei.

Background cytoplasmic signal in connective tissue and blood vessels.

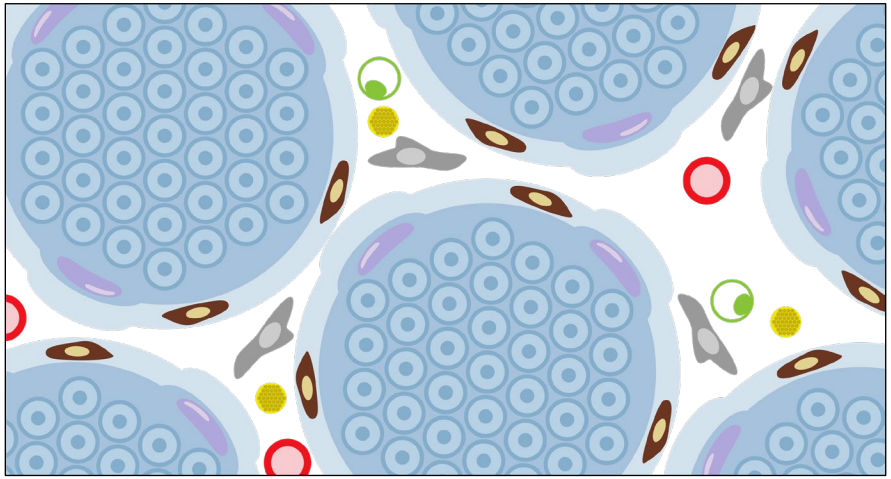
Results: Observation of stem cells in patient samples

Patient No. 1 Deltoid Muscle



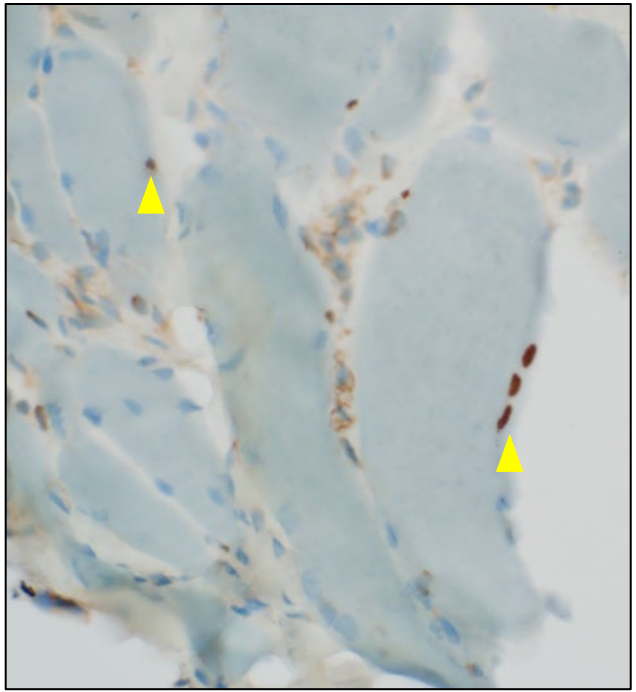
PAX7 Positive Stem Cells (Arrows)

Location of Stem Cells in Myofibers

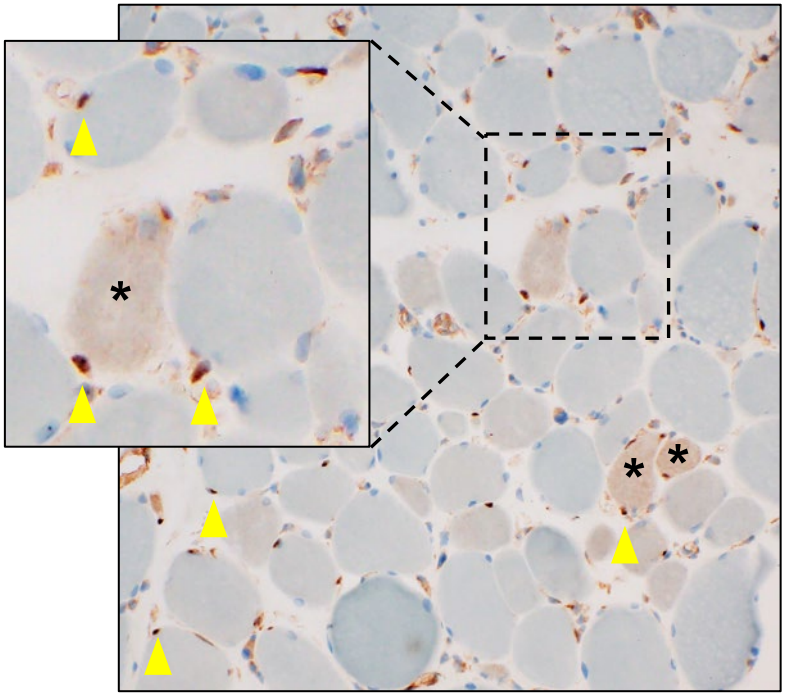


Results: Observation of stem cells in patient samples

Patient 1



Patient 3



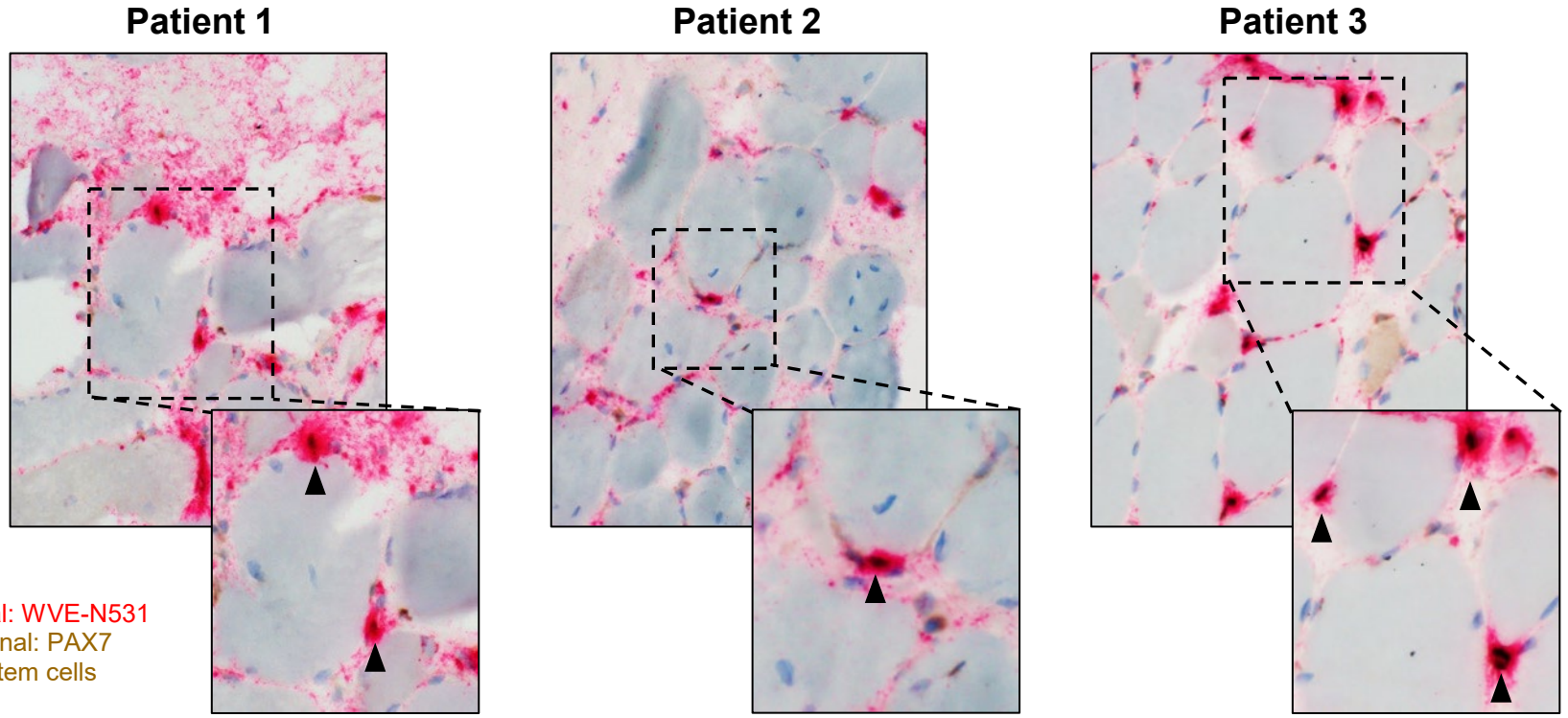
Note degenerating myofibers (asterisks) and PAX7 positive nuclei (arrowheads) surrounding these myofibers

PAX7 and RNAScope dualplex without probe

Controls: IgG for Pax7 – Negative, RNAScope Ubiquitin – Positive, & RNAScope DapB – Negative

Original mag: 20x and 40x

WVE-N531 demonstrated clear uptake in myogenic stem cells



Red signal: WVE-N531
Brown signal: PAX7
Positive stem cells

PAX7 and RNAScope dualplex

Controls: IgG for Pax7 – Negative, RNAScope Ubiquitin – Positive, & RNAScope DapB – Negative; Serial sections are not available; Original mag: 20x and 40x

Conclusions & next steps

- WVE-N531 demonstrated clear uptake in myocyte stem cells in all three patients as evaluated by dual PAX7 (stem cell marker) immuno-histochemistry and WVE-N531 RNAscope chromogenic assay.
- Stem cells were mostly associated with myofibers undergoing repair.
- Confirmation in larger cohort will be helpful.
- Dosing is underway in FORWARD-53: Phase 2 open-label clinical trial for boys amenable to exon 53 skipping, with data expected in 3Q 2024.

Acknowledgements

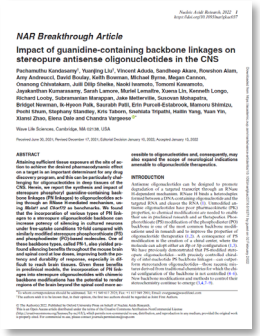
Thanks to all colleagues and contributors from Wave Life Sciences and our collaborators

Study participants & families

DMD Investigators

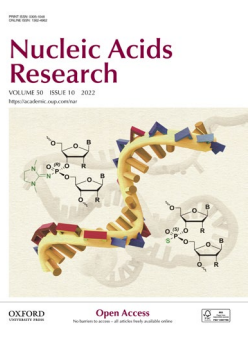
- Laurent Servais
- Craig Campbell
- Muath AlQurashi
- Mai Bader

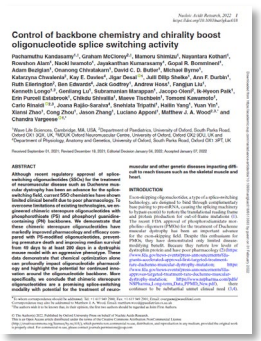
Silencing



Silencing

Splicing





RNA Editing