

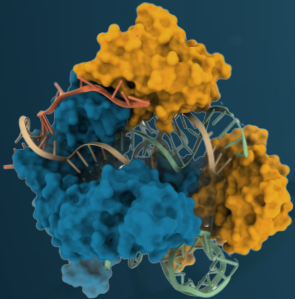


# Non-Human Primate Muscle Gene Editing Via Single Systemic AAV Delivery of an Ultra-Compact CRISPR Nuclease

Renan B. Sper, DVM, PhD.

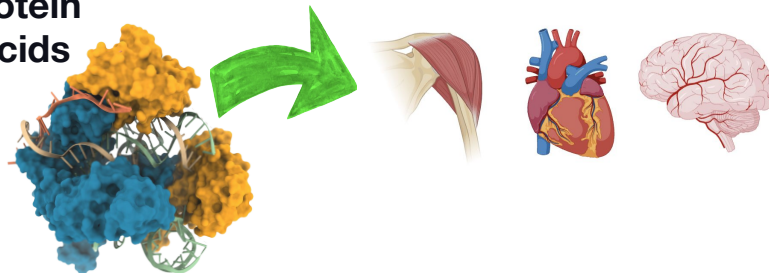
Principal Scientist, Preclinical Extrahepatic  
Program

ASGCT 28th Annual Meeting, May 2025  
New Orleans, Louisiana



# Mammoth CRISPR Systems Enable All-in-One AAV Delivery to Unlock Targets Beyond The Liver

NanoCas protein  
450 amino acids



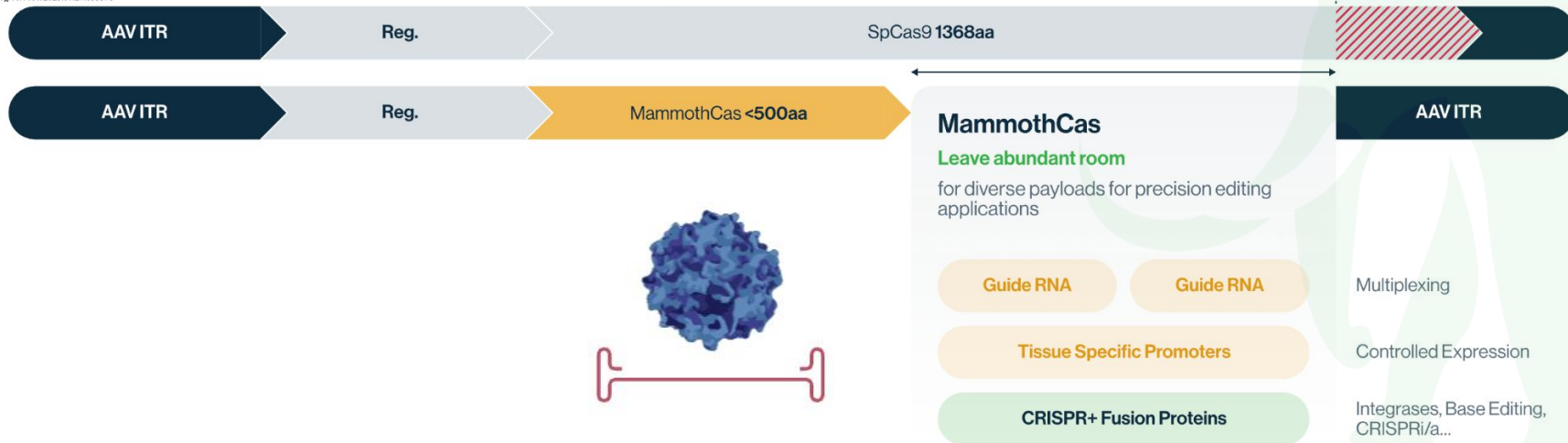
New Results

Follow this preprint

Single-AAV CRISPR editing of skeletal muscle in non-human primates with NanoCas, an ultracompact nuclease

Benjamin J. Rauch, Aaron DeLoughery, Renan Sper, Sean Chen, Sophia Yunanda, Megan Masnaghi, Ning Chai, Jason Chen, Lin Alexander Neckelmann, Ymer Bjornson, David Paez Espino, Alyssa Sancio, Christopher Schmitt, Clarissa Scholes, Ria Shah, Pooja Kyasandra Narendra, Sara Ansaloni, SuoZee Tan, Subhadra Jayaraman Rukmini, Sahana Somaiah, Shrivanti Suresh, Shikhi Minami, Stepan Tymoshenko, Will Wright, Siming Xu, James Broughton, Mazan Drory Retwitzer, Maggie Bobbin, Dave Yuan, Keith Abe, Mark DeWitt, Bohong Zhang, Lucas B. Harrington

doi: <https://doi.org/10.1101/2025.01.29.635576>



# DMD is a Severe Genetic Disease with Significant Unmet Medical Need



Proof-of-concept target for gene editing in skeletal muscle and heart

## Duchenne Muscular Dystrophy

X linked disease with childhood manifestation  
~1 in 5000 males birth worldwide

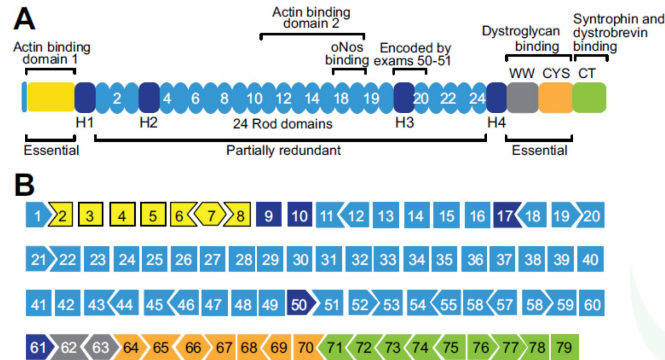
### DMD gene - Dystrophin protein

Progressive **muscle weakness**, severe **cardiorespiratory complications**

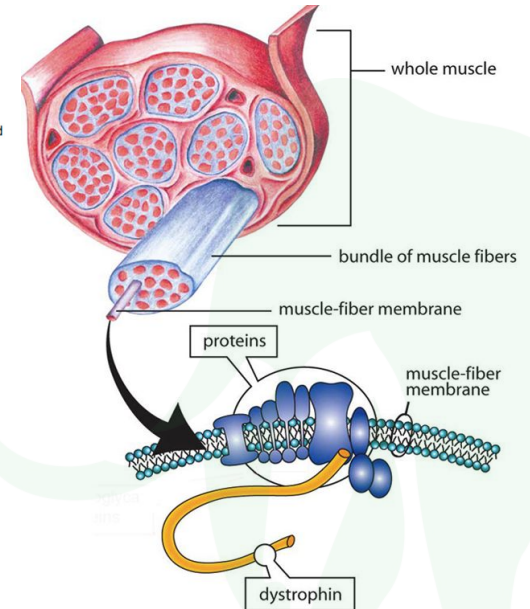
**Different mutations lead to truncation and lack of functional Dystrophin**

### Hypersensitive to injury

Initial rounds of injury and repair, followed by failed repair and muscle replacement by fat and fibrosis, compromising skeletal and cardiac function



Saad, Fawzy A., Gabriele Siciliano, and Corrado Angelini. "Advances in dystrophinopathy diagnosis and therapy." *Biomolecules* 13.9 (2023): 1319.



<https://www.mda.org/disease/duchenne-muscular-dystrophy/causes-inheritance>

# Opportunities for Gene Editing Compared to Standard of Care



## Standard of Care

### ASO

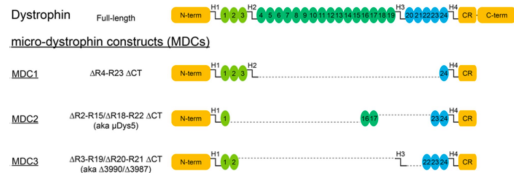
#### Exon skipping: partial phenotype restoration



Nguyen, Quynh & Yokota, Toshifumi, (2017), Immortalized Muscle Cell Model to Test the Exon Skipping Efficacy for Duchenne Muscular Dystrophy. J. Pers. Med., 7, 13, 10.3390/jpm7040013.

## 4 FDA-approved therapies

### AAV Gene Therapy (microdystrophin)



Choi, Eunyoung, and Taeyoung Koo. "CRISPR technologies for the treatment of Duchenne muscular dystrophy." *Molecular therapy* 29.11 (2021): 3179-3191.

## 1 FDA-approved therapy

## Limitations

Redosing

Biodistribution variability

Reduced cardiac intake (Morpholino)

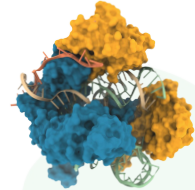
Potential nephrotoxicity (high, repetitive doses)

Biodistribution variability

AAV toxicity (liver and muscle immune response)

AAV genome dilution (muscle regeneration)

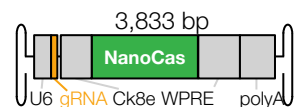
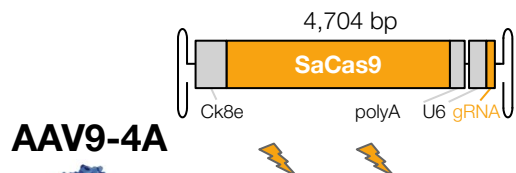
## Opportunities for Gene Editing



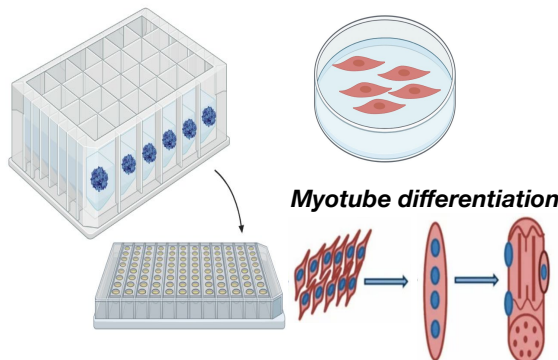
**Treat the underlying cause of the diseases (deletion, substitutions, insertion), restoring Dystrophin expression, potentially overcoming limitations of standard of care**

**Pre-clinical demonstration of multiple approaches and at least one ongoing clinical trial**

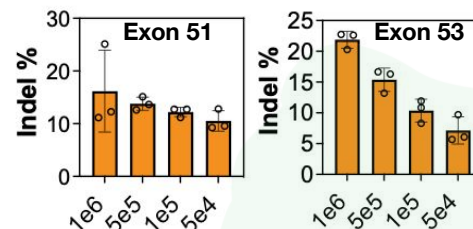
# In Vitro Transduction of AAV9-4A-NanoCas Results in *DMD* Indels in Human Myotubes



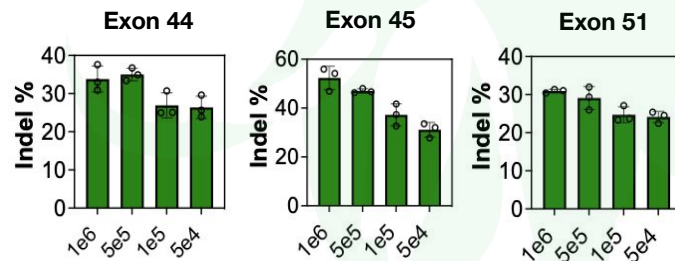
**In vitro transduction and indels analysis 30 days**



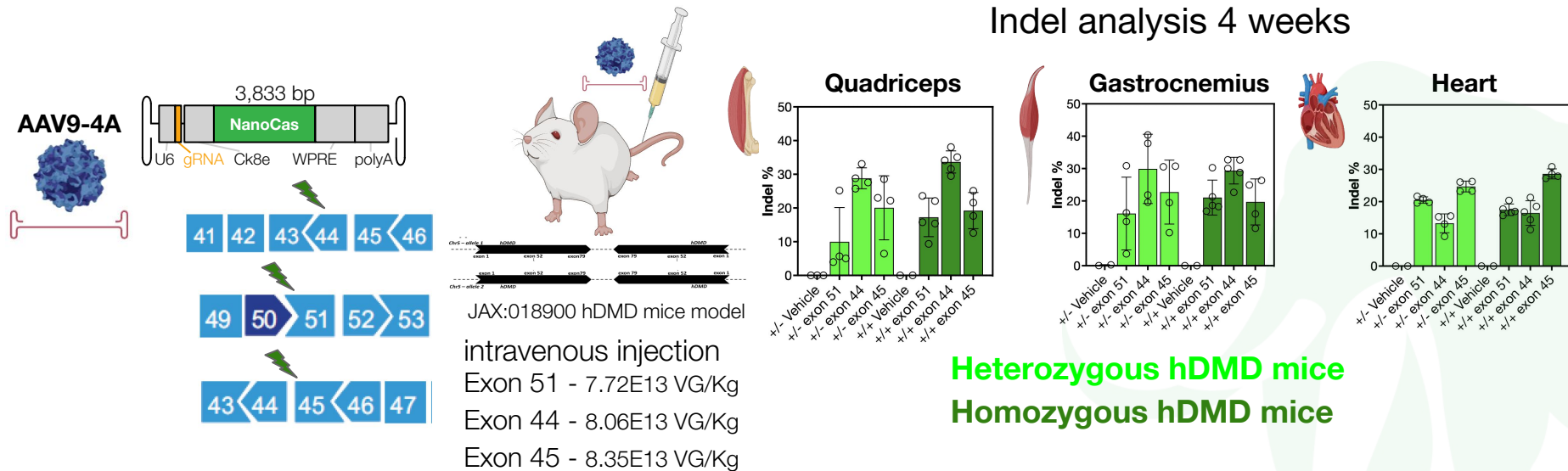
**SaCas9**



**NanoCas**



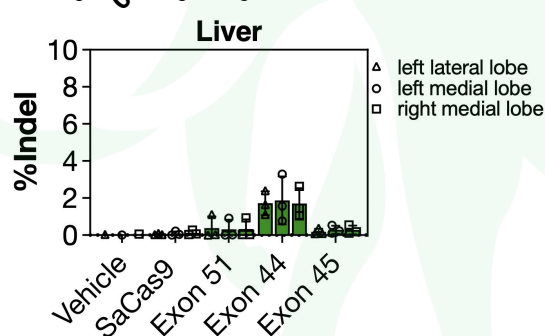
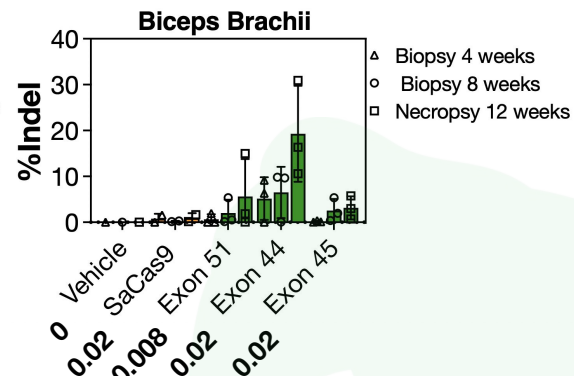
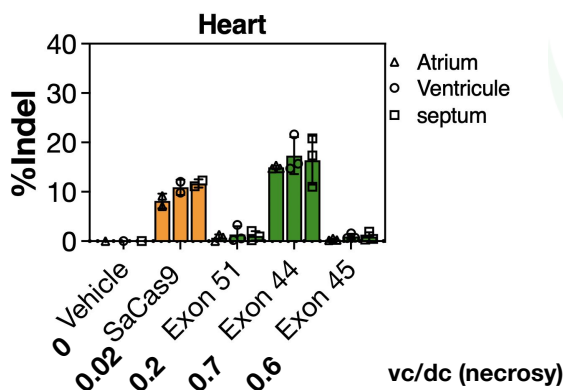
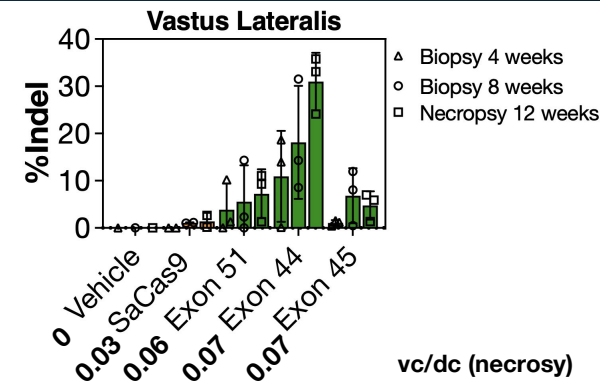
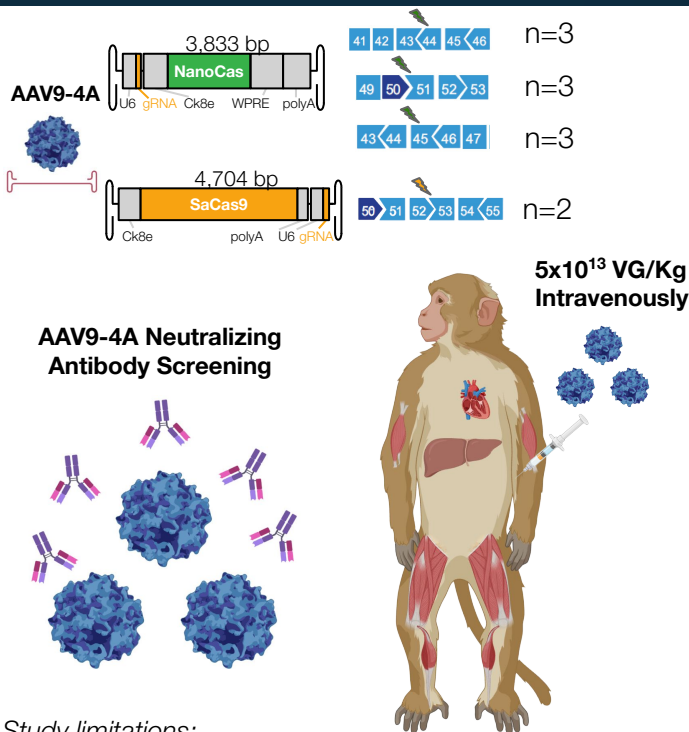
# Robust Editing in hDMD Mice Achieved with Intravenous Injection of AAV9-4A-NanoCas



*SaCas9 PAM mismatch in hDMD mouse model*



# Systemic Delivery of AAV9-4A-NanoCas Achieves Up to 40% Editing in NHP Cardiac and Skeletal Muscle



## Study limitations:

- Limited number of NHP animals
- Studies ongoing on collected NHP tissues to evaluate exon skipping



# Ultracompact NanoCas Flexes Its Editing In Muscle

- First demonstration of an **ultracompact gene editor** paired with a **muscle tropic capsid** showing robust **muscle editing in vitro, in mice, and NHP**
- NanoCas is approximately **half the size of other Cas systems** that are being tested in preclinical and clinical studies for DMD, **unlocking diverse gene modification approaches across multiple tissues**
- Our work complements others in the field for the scientific advancement of **genetic medicine approaches for extrahepatic indications**





# Acknowledgments



## Leadership team

Trevor Martin, CEO, co-founder  
Jennifer Doudna, co-founder SAB  
Janice Chen, CTO, co-founder  
Lucas Harington, CSO, co-founder  
Gabor Veres, SVP  
Elaine Sun, CFO and COO  
Siang Chin, General Counsel  
Aruna Perera, VP  
Rachel Herder, SVP  
Dave Kuo, SVP  
Jate Sam, SVP

## Preclinical team

Bohong Zhang  
Renan Sper  
Christopher Schmitt  
Jason Chen Lin

## Discovery and computational team

Ben Rauch  
Aaron DeLoughery  
Matan Drory  
Clarissa Scholes

## In vivo team

Keith Abe  
Megan Masnaghetti  
Siu Sze Tan  
Zach Wilson  
Sean Coakley

## Production and capability team (viral production)

Danni Frimannsson  
Aashish Lamichhane  
Winston Chang  
Ngan Nguyen

*In memory of Ning Chai*

## Assay development team

Maggie Bobbin  
Sara Ansaloni  
Subhadra Jayaraman  
Emel Alpaya

