Generating human-evolved, cell-type-specific AAV capsids for targeted gene delivery

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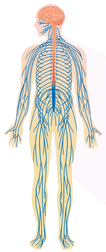
SANIA THERAPEUTICS

Sania pioneers ways to selectively target and safely control neural circuits as an access point to treating prevalent disorders



Neural circuits play an important role in many disease states. Targeting neural circuits enables treatments for many disease states across the body.

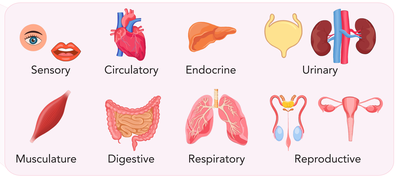


Figure 1. Our central and peripheral nervous system and example organ systems they innervate.

Our novel gene therapy approach combines precision gene delivery with a controllable therapeutic to build gene therapies capable of treating millions

Precision delivery

Cell-type specific AAV vector

Sania's R-Scan recreates human neural circuits to pioneer human-centric capsid evolution.

Circuit control

Therapeutic protein | Protein activator

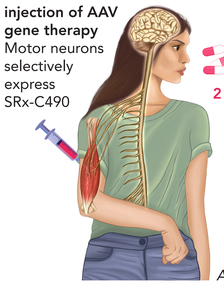
Delivered protein enables selective control of neural circuit with oral small molecule.

- Translatable R&D
- Enhanced safety
- Low dose
- Scalable & manufacturable

Abstract: 1606

Sania's gene therapies unlocks prevalent disorders such as spasticity

1 **Intramuscular injection of AAV gene therapy** Motor neurons selectively express SRx-C490



2 **Oral activator** for SRx-C490 to reduce hyperexcitability and treat spasticity in a titratable manner

Abstract: 1140

Figure 2. Sania's lead program in spasticity combines selective targeting of hyperexcitable motor neurons with neuromodulation via our therapeutic protein SRx-C490.

Sania's human-first capsid discovery pipeline maximises translatability

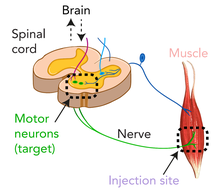
1 Libraries	2 Evolution	3 Ranking I	4 Gate I
Random mutagenesis and semi-rational design to build diversified AAV capsid libraries	R-Scan human directed evolution identifies novel capsids	Capsids are scored and ranked according to specific parameters	Targeted injection of lead capsids *in vivo* (mouse)

5 Ranking II	6 Gate II	7 SRX-T00X
Capsids ranked based on desirable properties e.g. known mechanism of action and no off target transduction	Targeted injection in NHP	Lead candidate used in toxicology and efficacy studies.

Figure 3. To maximise translatability we take a human-first approach to directed evolution, and have stages of ranking and testing capsids in animal models and human cell types.

2 R-Scan is an adaptable platform to evolve cell-type-specific AAV capsids. First use case, R-Scan-MN, evolves AAV capsids targeted to human motor neurons.

Target clinical circuit



Clinial circuit recreated in R-Scan-MN with human neurons in vitro

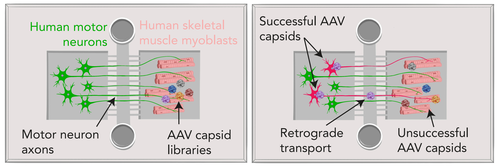


Figure 4. We use Sania's R-Scan platform to recreat clinically relevant neural circuits with human neurons in a microfluidic system. R-Scan-MN is used to identify AAV capsids that efficiently tranduce motor neurons after intramuscular injection.

R-Scan platform evolves novel AAV capsids in human neural ciruits. Our initial work has focussed on evolving AAVs that efficiently tranduce motor neurons after intramuscular injection.

3 Sania's human-evolved AAV capsids show superior transduction of human IPSC-derived motor neurons and human neuromuscular organoids

Human motor neurons *in vitro*

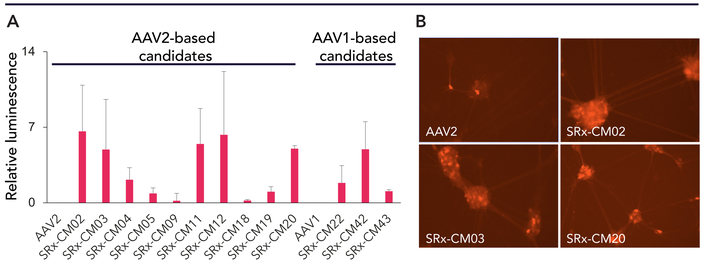


Figure 5. Our novel AAV vectors are tested for their ability to transduce human motor neurons in vitro. (A) Luciferase assay measuring protein expression in human motor neurons of a selction of our AAV1 and AAV2-based candidates. ***(B)*** Example images of human motor neurons transduced with AAV vectors encoding a red fluorescent protein.

Human neuromuscular organoids

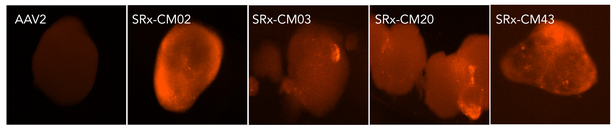
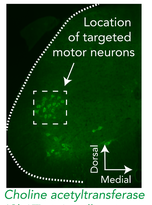


Figure 6. To test our capsids in a human system that more closely mimics the in vivo situation we test our AAV capsids in human neuromuscular organoids. Example images of organoids are shown transduced with either an AAV2 control or Sania vectors all encoding a red fluorescent protein.

4 Intramuscular injection of Sania's vectors results in highly efficient and selective motor neuron transduction

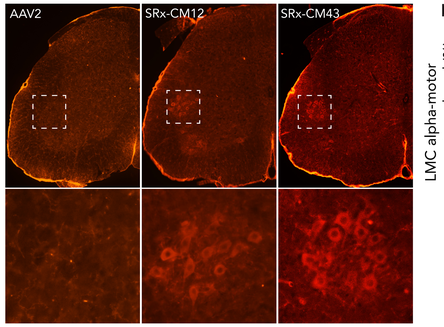
A *Single intramuscular injection*

B *Spinal cord anatomy*



Choline acetyltransferase (ChAT) stains all motor neurons

C



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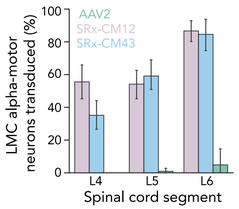

Figure 7. Sania's novel vectors are highly efficient at transducing motor neurons in vivo after intramuscular injection in mouse. (A) A single intramuscular injection was performed into the gastrocnemius muscle. ***(B)*** Spinal cord anatomy allows us to locate the location of motor neurons innervating that muscle. ***(C)*** Example images of spinal cord motor neurons transduced with the AAV vector expressing a red fluorescent protein. Comparing Sania's vectors with an AAV2 control. ***(D)*** Quantification of motor neuron transduction. LMC = lateral motor column.

5 Vectors show minimal biodistribution as well as detargeting from the muscle

Vector biodistribution following intramuscular injection

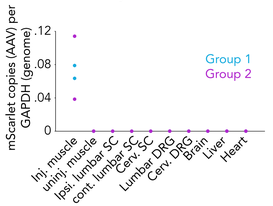

Figure 8. Biodistribution of AAV following intramuscular injection in mouse. Groups refer to two separate mixtures of injected AAVs. In total, we tested six Sania vectors and two wildtype controls. Using qPCR, vector was found in the injected muscle but not in other tissues.

Identification of vectors de-targeted from the muscle

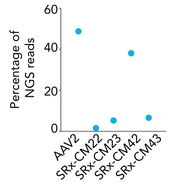

Figure 9. Next generation sequencing was used to identify the vectors which had transduced the injected muscle tissue. We identified several vectors that show efficient motor neuron transduction and limited muscle transduction.

5 Sania's motor neuron targeted AAV capsids have several mechanisms of action

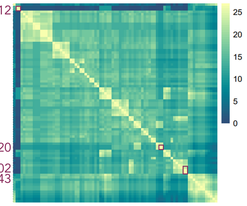

Figure. 10. Heatmap plotting the reverse Hamming distance between selected capsids and their original parental library. Reverse Hamming is the count of identical positions in the alignment between the peptide sequences. Selected candidates have been highlighted showing that their peptide insertions fall within different alignment clusters and consequently using different mechanisms of action.

Conclusions, ongoing work & next steps

- **Conclusions:** Using our R-Scan platform, we have generated human-evolved motor neuron specific capsids that are highly effective in human motoneurons, human neuromuscular organoids and in vivo in mouse.
- **Ongoing work:** We are currently validating our novel capsids in the NHP.
- **Next steps:** We are using R-Scan platform to develop sensory neuron specific capsids that will be used for other indications.

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