Bringing Gene therapy based SOD1 silencing towards clinical trials

A highly efficacious, off-target free and biomarker supported strategy for familial ALS

Joseph Scarrott
Study aims
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1. Evaluate the *in vivo* efficacy of SOD1 silencing in the SOD1-G93A mouse model by a clinic ready vector.
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2. Measure CSF SOD1 protein levels as a biomarker of effective dosing and efficacy of SOD1 knockdown.
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1. Evaluate the *in vivo* efficacy of SOD1 silencing in the SOD1-G93A mouse model by a clinic ready vector.

2. Measure CSF SOD1 protein levels as a biomarker of effective dosing and efficacy of SOD1 knockdown.

3. Investigate miRNA-like sequence specific off-target effects.
Background

RNA interference as a strategy for SOD1-fALS gene therapy.

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Extended survival has been demonstrated by:

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Extended survival has been demonstrated by:
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- AAV delivery of shRNA
- AAV delivery of artificial miRNA

Background

RNA interference as a strategy for SOD1-fALS gene therapy.

Extended survival has been demonstrated by:
- Lentivirus delivery of shRNA
- AAV delivery of shRNA
- AAV delivery of artificial miRNA
- Antisense oligonucleotide therapy

Clinic-ready vector design
Clinic-ready vector design
In Vivo Efficacy
Route of injection

CB
SC
BS
HC
Ctx
Route of injection

Post-natal Day:
1
35
40
75
120

Assess motor performance
Onset of visible symptoms
Substantial distress
Death endpoint
Route of injection

Cerebellum

Cisterna magna

Cortex

Post-natal Day: 1 35 40 75 120

Assess motor performance
Onset of visible symptoms
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Cortex
Cerebellum
Cisterna magna

CB
BS
Ctx
HC
SC
## P1 – neuroscoring and onset

<table>
<thead>
<tr>
<th>Neuroscoring Assessment</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>0.5</td>
</tr>
<tr>
<td>Abnormal gait</td>
<td>1</td>
</tr>
<tr>
<td>Severe “waddle”</td>
<td>1.5</td>
</tr>
<tr>
<td>Dragging one hind limb</td>
<td>2</td>
</tr>
<tr>
<td>Paralysis of one hind limb</td>
<td>3</td>
</tr>
<tr>
<td>Moribund</td>
<td>4</td>
</tr>
</tbody>
</table>
P1 – neuroscoring and onset

Neuroscoring Assessment

| Abnormal gait | 1 |
| Severe “waddle” | 1.5 |
| Dragging one hind limb | 2 |
| Paralysis of one hind limb | 3 |
| Moribund | 4 |

Onset

- scAAV9-hSOD1ssi
- scAAV9-hSOD1si

Graph showing neuroscoring and onset over time in days.
P1 – neuroscoring and onset

- scAAV9-hSOD1ssi
- scAAV9-hSOD1si

![Graph showing neuroscoring over time](image-url)
P1 – neuroscoring and onset

- scAAV9-hSOD1ssi
- scAAV9-hSOD1si

Neuroscoring vs Time (days)

Onset (20% drop in rotarod) vs Time (days)
P1 – rotarod and survival
P1 – rotarod and survival

Latency to fall (seconds)

Vector delivery

Time (days)
P1 – rotarod and survival

Vector delivery

Latency to fall (seconds)

Survival (%)
P1 – rotarod and survival

Latency to fall (seconds)

Vector delivery

Time (days)

Survival (%)

42% increase
P1 mice – 144 days old
Treatment rescues motor neurons

shRNA  Control

Motor neurons per section

scAAV9-siSOD1  scAAV9-ssiSOD1

*
Treatment reduces reactive gliosis

shRNA

Control

Purple = GFAP  Orange = Ibal
P40 – neuroscoring and rotarod
P40 – neuroscoring and rotarod

![Graph showing neuroscoring over time for AAV9-hSOD1ssi and AAV9-hSOD1si](image)
P40 – neuroscoring and rotarod

Neuroscoring

Time (days)

Latency to fall (seconds)

AAV9-hSOD1ssi

scAAV9-hSOD1ssi

Vector delivery

Time (days)
P40 – survival

14% increase in median survival
Measurement of SOD1 depletion in the CSF
Cerebrospinal Fluid

ng hSOD1/µg total protein

scAAV9-siSOD1  |  scAAV9-ssiSOD1

↓ 63%

The University Of Sheffield.
In vitro investigation of off-target effects.
Seed regions in 3’UTRs

3’UTR Seed region frequency

- High
- Medium
- Low
On-target mRNA

RISC

Knockdown due to perfect complementarity

RISC

No knockdown due to sequence mismatch
On-target mRNA

RISC

Knockdown due to perfect complementarity

RISC

No knockdown due to sequence mismatch

Off-target mRNA

RISC

Repression of off-target mRNA due to seed region match

RISC

Repression of off-target mRNA due to seed region match despite central mismatch
## Construct validation

<table>
<thead>
<tr>
<th>Construct</th>
<th>SOD1 Targeting?</th>
<th>Seed Region</th>
</tr>
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<tbody>
<tr>
<td>Therapeutic construct</td>
<td>YES</td>
<td>CATGAAC</td>
</tr>
<tr>
<td>Unrelated shSOD1</td>
<td>YES</td>
<td>CAGTCAG</td>
</tr>
<tr>
<td>Mismatch control</td>
<td>NO</td>
<td>CATGAAC</td>
</tr>
<tr>
<td>Negative control</td>
<td>NO</td>
<td>None</td>
</tr>
</tbody>
</table>

![Bar chart showing fold change for different constructs](chart.png)

- **Therapeutic vector**
- **Unrelated shSOD1**
- **Mismatch control**
- **Negative control**
- **Mock**
Genes differentially expressed from Negative control

- 1 shared downregulated gene: SOD1

Venn diagram:
- Therapeutic vector: 0 shared downregulated genes
- Mismatch control: 0 shared downregulated genes
- Unrelated shSOD1: 1 shared downregulated gene

(13%)
(20%)
(11.5%)
Summary

• The clinic ready vector is effective at prolonging lifespan and ameliorating disease in a mouse model of ALS

• The therapeutic shRNA appears to be specific to SOD1 mRNA with no noticeable sequence-specific off-target effects

• Measurement of SOD1 depletion in the CSF of treated patients can be a simple and effective biomarker of treatment efficacy
Acknowledgements

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Thank you for your attention!