Phase I Gene Therapy Trial for SMA
Delivering the Survival Motor Neuron Gene
by scAA9
RAC Protocol 1210-1189

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Presentation Objectives

- Present study goals & rationale for clinical trial
- Describe pre-Clinical studies in preparation for SMA gene therapy trial
- Present the toxicology safety studies completed and planned in preparation for clinical trial
- Summarize the clinical protocol
- Address any unanswered questions
SMA Type 1

Acute Werdnig-Hoffman

- SMA refers to a group of disorders characterized by loss of motor neurons that map to 5q

- SMA type 1 most common genetic cause of infant mortality; Onset ≤ 6 mos with signs of weakness by 3 mo in 95% of cases

- Severe hypotonia, progressive limb and bulbar muscle weakness; Weak cry, poor suck & respiratory failure with Death by 2 yo
Pathogenesis

• SMA is caused by homozygous mutations of Survival Motor Neuron (SMN1) gene (telomeric) & retention of at least one copy of nearly identical (centromeric) gene - SMN2

• SMN1 produces full length transcript
• SMN2 produces alternatively spliced mRNA lacking exon 7 (SMNΔ7)
  - SMNΔ7 is unstable and rapidly degraded
  - Low levels full-length functional SMN are produced
SMA is caused by loss of SMN1 but retention of SMN2

- The more SMN2 mRNA, the more full length protein and the milder the disease (i.e., SMA types 2 & 3)
## Classification of SMA Correlation with SMN Copies

<table>
<thead>
<tr>
<th>Type</th>
<th>Age of Onset</th>
<th>Age at Death</th>
<th>SMN2 Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Prenatal</td>
<td>&lt;1 mo</td>
<td>1</td>
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<tr>
<td>1</td>
<td>0-6 months</td>
<td>&lt; 2yrs</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 18 months</td>
<td>&gt;2yrs</td>
<td>3,4</td>
</tr>
<tr>
<td>3</td>
<td>18 months- 3 years</td>
<td>Adult</td>
<td>3,4</td>
</tr>
<tr>
<td>4</td>
<td>21 years</td>
<td>Adult</td>
<td>4-8</td>
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</tbody>
</table>
Multiple lines of Evidence support precipitous loss of motor neurons in SMA type 1

- Linear drop in motor neurons (CMAP) correlates with motor assessment (CHOP INTEND) Finkel 2012
- Similar findings by Swaboda et al 2005 correlating MUNE with CMAPs and function
Alternative Treatments

1) Various strategies have been tried without success
   Small molecules found to induce SMN in culture but not in clinical trials [Valproic acid (Kissel 2011) and Phenylbutyrate (Mercuri 2007)]

2) High throughput screening has identified small molecules that increase SMN levels
   None tested yet in clinical trials; most effective in animals and are in development

3) Antisense Oligonucleotide (AON) alters SMN2, & restores splicing producing full length mRNA and functional protein; AON requires intrathecal delivery; Clinical trial results not yet reported.
Pre Clinical Data

• \(\Delta 7\)SMA mouse model (SMN\(2^{+/+}\); SMN\(\Delta 7^{+/+}\);Smn\(^{-/-}\))
  - Developed at OSU in Burghes Lab
• Develops phenotype of SMA
  - Normal at birth followed by rapid onset and progression with death at 13-16 days (CMAP decline occurs as well)
• IV scAAV9.CB.SMN extends survival to 1 year in P1 mice receiving 3.3 X 10\(^{14}\) vg/kg in facial vein (Foust et al 2010)
Pre Clinical Data

• scAAV9 delivery to mouse following IV injection (dose $3.3 \times 10^{14}$)

• Time Dependent Transduction
  - 1-2 day old mice targeted $\geq 70\%$ of MN
  - Loss of motor neuron targeting occurred within the first 10 d
Pre Clinical Data

- Therapeutic benefit dose and time dependent
- P1 High Dose shows prolonged survival
- A five-fold reduction in dose (6.7X $10^{13}$ vg/kg) reduces survival to 35 days
- Treatment at P5 results in survival to 28 days
- Treatment at P10 had no effect
Extending Pre Clinical Data to Non-Human Primates

- Cynomolgus macaques IV dose $1-3 \times 10^{14}$ vg/kg showed motor neuron targeting ($\geq 70\%$ transduced) following gene delivery in first 90 days.
Message from Pre-Clinical

• Treatment effect has greatest impact with early gene replacement!
  - Early introduction of SMN is a biological requirement for \( \Delta 7 \) SMA Mouse survival
  - True also for SMA mouse model with inducible SMN (Le 2011; Lutz 2011)

• Efficacy limited dosing range that must be considered for clinical trials!
GLP Pharm-Tox-Biodistribution Study for SMN Gene Replacement

<table>
<thead>
<tr>
<th>Treatment (vg/kg)</th>
<th>Procedure</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
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<tbody>
<tr>
<td>Facial v. inj.</td>
<td></td>
<td>0</td>
<td>6.7 x 10(^{13})</td>
<td>3.3 x 10(^{14})</td>
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<tr>
<td>Week 3</td>
<td>CBC/Chem</td>
<td>5M/5F</td>
<td>5M/5F</td>
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<td>Coagulation</td>
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<td>Antibodies (AAV9 and SMA)</td>
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<td>Histopathology</td>
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<td>Biodistribution</td>
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<td>Expression</td>
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<td>CBC/Chem</td>
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<td>Coagulation</td>
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Non-GLP Toxicology Studies

- **Mice** (n = 14) treated IV with 3.3 X 10^{14} (high dose) scAAV9.CB.SMN at day 1; mice sac’ed at 24 weeks with no toxicity

- **Cynomolgus macaques** (n = 4) treated with intermediate dose (scAAV9.CB.SMN 6.7 X 10^{13} vg/kg) at day 90 simulating clinical trial and sac’ed at 36 weeks
Non-GLP Monkey Studies

- Hematology and clinical chemistry values normal
- Organ system histology was normal

Binding Ab Titers at 6 months post-injection

<table>
<thead>
<tr>
<th>Monkey ID</th>
<th>Anti-AAV9</th>
<th>Anti-SMN</th>
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<tbody>
<tr>
<td>11C2</td>
<td>1:25,600</td>
<td>NEG</td>
</tr>
<tr>
<td>11C11</td>
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<td>11C14</td>
<td>1:12,800</td>
<td>NEG</td>
</tr>
<tr>
<td>11C16</td>
<td>1:25,600</td>
<td>NEG</td>
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Taking this to clinical trial
Target Population for Clinical Trial
SMA type 1

**We believe that potentially high risk procedure should not be done in stable population until efficacy established**
Clinical Protocol

- Single-site, Phase I Gene Transfer trial in SMA type 1
- N = 9 clinically affected subjects, age 6 mo. or younger, proven SMN mutation with 2 copies of SMN2
- Exclusion criteria:
  - Use of ventilatory support or pulse oximetry <95% saturation
  - Other organ system disease, active viral infection
  - Ongoing IS therapy
  - Anti-AAV9 Ab titer ≥1:1600 ELISA
- scAAV.CB.SMN will be delivered intravenously through a venous catheter in the intensive care unit at Nationwide Children’s Hospital.

![Gene Transfer Diagram]
Clinical Protocol

• Dose escalation study
  - Cohort 1 (Low Dose)  $6.7 \times 10^{13}$ vg/kg (n=3)
  - Cohort 2 (Mid Dose)  $2.2 \times 10^{14}$ vg/kg (n=3)
  - Cohort 3 (High Dose)  $3.3 \times 10^{14}$ vg/kg (n=3)

• Primary outcome measure: Safety

• Secondary outcome measures:
  - CHOP INTEND (Head control, righting reflexes, truncal movements, rolling, ventral suspension, standing)
  - Time to invasive ventilation

• Follow up at 1, 2, 4, 8, 12 weeks; every three months 2 yrs
Participants/Research Team

- Brian K. Kaspar, PhD
- Arthur Burghes, PhD
- Kevin Foust, Ph.D
  - Research Scientists
- K. Reed Clark, Ph.D
  - Virologist/Director of Vector Manufacturing Laboratory NCH
- John T. Kissel, MD
  - SMA specialist
- Richard Shell, MD
  - Pulmonologist