Disclosures  Arthur Burghes

SAB AveXis

Performed studies funded by AveXis

Consulted for Novartis
<table>
<thead>
<tr>
<th>Type of Biomarker</th>
<th>Definition</th>
<th>Actual or Potential Examples in SMA</th>
<th>Validation Dependent Upon Effective Therapy?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognostic</td>
<td>Predicts a future clinical outcome</td>
<td>SMN2 copy number → Disease severity</td>
<td>No</td>
</tr>
<tr>
<td>Disease Progression</td>
<td>Identifies the severity of disease impact</td>
<td>Compound muscle action potential amplitude → Motor neuron loss SMA-MAP panel</td>
<td>No</td>
</tr>
<tr>
<td>Predictive</td>
<td>Predicts a future clinical response to therapy and helps stratify therapies</td>
<td>Reduced CMAP amplitude → Less response to SMN restoring therapies</td>
<td>Yes</td>
</tr>
<tr>
<td>Pharmacodynamic</td>
<td>Monitors or quantifies a therapeutic effect</td>
<td>Increased full-length SMN transcripts</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased SMN protein →Effective induction of SMN2 gene</td>
<td></td>
</tr>
<tr>
<td>Surrogate Endpoint</td>
<td>Predicts a future clinical response to therapy and a change in the endpoint</td>
<td>Increased MUNE → Improved physical function</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>is associated with the future clinical response</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Types of Biomarkers. Compound Muscle Action Potential (CMAP), Motor Unit Number Estimation (MUNE), SMA-MAP panel of biomarkers developed by the SMA-Foundation which correlate with the Hammersmith motor function scale

Arnold et al. Development and Testing of Biomarkers in SMA. *in Spinal Muscular Atrophy* 
Prognostic Biomarker *SMN2* copy number

B. American population Swoboda and Prior

D. Combined data
Points on SMN2 prognostic

1) SMN2 copy number exceptions between type 1 (2 copies of SMN2) and Type 2 (3 copies of SMN2) are relatively rare (5-10%) of type 2 cases have 2 copies of SMN2. Type 1s with 3 copies of SMN2 do have early onset but seem to have milder progression. " modifier of onset

2) The variant SMNG859C increases the amount of full length SMN mRNA. The variant SMNG859C has a frequency of .00348 in SMN2 genes in the ExAC data base so is a rare variant. In the Spanish population (US) it accounts for 50% of the type 2 and 3 case with 2 copies of SMN2. (Bernal et al J Med Genet 47: 640-642, 2010, Prior, T. W. et al Am J Hum Genet 85, 408-413 2009)

3) All cases diagnosed before the age of 6 months with symptoms and two copies of SMN2 have type 1 SMA.

4) The variant SMNG859C is not found in type 1 SMA.

5) The majority of type 2 cases have 3 copies of SMN2 but 50% of type 3s also have 3 copies. When the type 3 cases are divided into 3a and 3b the milder case have 4 copies of SMN2.
SMA-MAP Analyte Panel correlate with muscle function

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Visit-Group Interaction F-test</th>
<th>Group F-test</th>
<th>Analyte</th>
<th>Visit-Group Interaction F-test</th>
<th>Group F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein B</td>
<td>p= 0.287</td>
<td>p= 0.141</td>
<td>IGFBP6 *</td>
<td>p= 0.054</td>
<td>p= &lt;0.001</td>
</tr>
<tr>
<td>AXL Receptor Tyrosine Kinase</td>
<td>p= 0.265</td>
<td>p= &lt;0.001</td>
<td>Leptin</td>
<td>p= 0.918</td>
<td>p= 0.124</td>
</tr>
<tr>
<td>C-Reactive Protein</td>
<td>p= 0.492</td>
<td>p= 0.895</td>
<td>Monocyte Chemotactic Protein 1</td>
<td>p= 0.444</td>
<td>p= 0.322</td>
</tr>
<tr>
<td>Cadherin-13 *</td>
<td>p= 0.727</td>
<td>p= 0.019</td>
<td>Myoglobin *</td>
<td>p= &lt;0.001</td>
<td>p= 0.613</td>
</tr>
<tr>
<td>COMP *</td>
<td>p= 0.216</td>
<td>p= &lt;0.001</td>
<td>Osteopontin</td>
<td>p= 0.064</td>
<td>p= 0.182</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>p= 0.807</td>
<td>p= 0.686</td>
<td>Peptidase D *</td>
<td>p= 0.01</td>
<td>p= &lt;0.001</td>
</tr>
<tr>
<td>C1qR1 *</td>
<td>p= 0.466</td>
<td>p= &lt;0.001</td>
<td>Placenta Growth Factor</td>
<td>p= 0.418</td>
<td>p= 0.113</td>
</tr>
<tr>
<td>CFHR1</td>
<td>p= 0.825</td>
<td>p= 0.557</td>
<td>Serum Amyloid P-Component</td>
<td>p= 0.529</td>
<td>p= 0.2</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV *</td>
<td>p= 0.468</td>
<td>p= &lt;0.001</td>
<td>Tenascin-X</td>
<td>p= 0.985</td>
<td>p= 0.243</td>
</tr>
<tr>
<td>Endoglin</td>
<td>p= 0.484</td>
<td>p= 0.002</td>
<td>Tetranectin *</td>
<td>p= 0.283</td>
<td>p= &lt;0.001</td>
</tr>
<tr>
<td>Fetuin A</td>
<td>p= 0.399</td>
<td>p= 0.861</td>
<td>Thrombospondin-4</td>
<td>p= 0.512</td>
<td>p= &lt;0.001</td>
</tr>
<tr>
<td>Fibulin-1C</td>
<td>p= 0.358</td>
<td>p= 0.591</td>
<td>Chitinase-3-like Protein 1 *</td>
<td>p= 0.009</td>
<td>p= &lt;0.001</td>
</tr>
<tr>
<td>HER2</td>
<td>p= 0.466</td>
<td>p= 0.021</td>
<td>Kolb et al in preparation</td>
<td>NeuroNext</td>
<td></td>
</tr>
</tbody>
</table>

Multi-Analyte Panel Summary

Analytes that changed over time differently in the healthy and SMA (SMN2 = 2) cohorts

- IGFBP6
- Myoglobin
- Osteopontin
- Peptidase D
- Chitinase-3-like Protein 1

(Kolb et al in preparation)

Analytes that DID NOT change over time differently in the healthy and SMA (SMN2 = 2) cohorts, however had significantly (p= <0.05) different levels between groups

- AXL Receptor Tyrosine Kinase
- Cadherin-13
- COMP
- C1qR1
- Dipeptidyl peptidase IV
- Endoglin
- HER2
- Tetranectin
- Thrombospondin-4

Other serum biomarkers Micro RNAs

Serum levels of miR 9 and 132 altered in levels between healthy age matched controls and SMA. No significant correlation to motor function scale

(Catapano et al. Mol Ther Nucleic Acids. 2016 5(7):e331.)

but which if any of these markers responds to therapy?

Only have data in mice for change with therapy.
SMA-MAP and Biomarkers panel analytes that can be tested in mice and are altered in SMA mice

IGF levels at PND7 in treated and untreated Taiwanese SMA mice

Suzan M. Hammond et al. PNAS 2016;113:10962-10967

SMA-MAP Serum markers altered at P12 in delta 7 SMA mice and responsive to ASO therapy delivered at P0. 5 out of ten markers tested Responded.

Arnold et al Plos one in Press

miR132 response to treatment at P0 measured at P2.

Catapano et al. Mol Ther Nucleic Acids. 2016 5(7):e331
Longitudinal Measures of SMN-corrected biomarkers. Delta 7 SMA mice treated at P0 with ASO and then the biomarker followed with blood samples. DPPIV and fetuin A showed no statistically significant change compared to ASO-Het and Het mice at either P30 or P90. The other 3 analytes, tetranectin, osteopontin, and vitronectin, showed no change at P30 but were all altered at P90. *<0.05, **<0.01. >? Respond to SMN levels decaying.

Table 3: Biomarkers in SMA Mice, SMN-restored Mice, and Human SMA

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Change in SMA mice</th>
<th>Normalized with SMN</th>
<th>Correlation with MHFMS (Kobayashi et al. 2013)</th>
<th>Change in SMA (&lt;6 months) (Kolb et al. 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopontin</td>
<td>↑</td>
<td>Yes</td>
<td>Direct</td>
<td>No Δ</td>
</tr>
<tr>
<td>DPPIV</td>
<td>↑</td>
<td>Yes</td>
<td>Direct</td>
<td>↑</td>
</tr>
<tr>
<td>Tetranectin</td>
<td>↑</td>
<td>Yes</td>
<td>Direct</td>
<td>↑</td>
</tr>
<tr>
<td>Fetuin A</td>
<td>↓</td>
<td>Yes</td>
<td>Inverse</td>
<td>No Δ</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>↓</td>
<td>Yes</td>
<td>Inverse</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

Arnold et al Plos One 2016 in press
Pharmacodynamic Markers

1) Both SMN protein and full length SMN levels should a wide variation of levels in blood and do not correlate with muscle function score or SMN2 copy number. Not a good prognostic marker.

2) Increased full length SMN mRNA in blood can be used if therapeutic agent gets there. Measure increase of full length from initial sample taken. Shows treatment effects SMN2 ability to produce full length SMN. Same can be applied to SMN protein. Increased full-length SMN transcripts, Increased SMN protein→Effective induction of SMN2 gene

3) Increased full length SMN mRNA in CSF could be measured but not reported.

4) Increased SMN levels in CSF in trial of nusinersen. CSF samples in the 9-mg group the baseline SMN levels were, $0.31 \pm 0.18$ pg/mL; after treatment the levels were $0.59 \pm 0.22$ pg/mL; $p = 0.06$; 161% mean increase. Using a sensitive ELISA. Indicates a trend but not significant. (Ciriboga et al. Neurology. 2016 Mar 8;86(10):890-7)
Compound muscle action potential CMAP. Type 1 SMA above 1mV on start of treatment predict strong response.

Once motor neurons lost can only get benefit from remaining. 

Finkel et al 2013 Neuromuscul Disord. (2):112-5
Intrathecal injection of scAAV9-shRNA in 5 day old piglets to create large animal model of SMA what does it predict

Time course of symptoms progression

PND5
injection
PND24-34
1st sign of weakness
Splayed gait
PND33-47
Mainly sitting
PND38-55
Mainly crawling
PND46-69
sacrifice

Duque et al Ann Neurol. 2015 77(3):399-414
Transduction profile in scAAV9-SMN treated piglets

<table>
<thead>
<tr>
<th>Group</th>
<th>number of animal</th>
<th>Age at sacrifice</th>
<th>Immunosuppressive treatment</th>
<th>Age at injection</th>
<th>Dose vector (vg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>79 ± 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>shRNA</td>
<td>5</td>
<td>61 ± 7</td>
<td>Prograf</td>
<td>PND5</td>
<td>6.5x10^{12}</td>
</tr>
<tr>
<td>SMN early</td>
<td>5</td>
<td>71 ± 2</td>
<td>Prograf + Cellcept</td>
<td>PND5 PND6</td>
<td>6.5x10^{12} 8x10^{12}</td>
</tr>
<tr>
<td>SMN late</td>
<td>5</td>
<td>73 ± 3</td>
<td>Prograf + Cellcept</td>
<td>PND5 PND33-36</td>
<td>6.5x10^{12} 8x10^{12}</td>
</tr>
</tbody>
</table>

(Prograf = tacrolimus, FK506. Cellcept = Mycophenolate mofetil. Block T-cell response)

Spinal cord section from lumbar 6 segment stained for GFP and human SMN (human specific antibody)

Duque et al Ann Neurol. 2015 Mar;77(3):399-414
Control

Early rescue
Late rescue

No rescue
Correlation of MUNE, CMAP and Motor Neuron loss with SMN restoration in the pig.

Graphs showing CMAP and MUNE measurements for early and late rescue groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fibrillation</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>shRNA (5 pigs)</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Control (6 pigs)</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Motor neuron path also improved.

Ann Neurol. 2015 Mar;77(3):399-414
Summary

1) SMN2 copy number is a prognostic marker. 2 copies of SMN2 and onset before 6 months does indicate type 1 SMA. 3 copy type 1 SMA cases do occur and are milder but have an earlier onset.

2) There are serum biomarker panels available that correlate with motor function scores and are changes in SMA in the NeuroNext trial.

3) The amount of blood full length SMN mRNA or SMN protein does not correlate with SMN2 copy number or motor function score.

4) The level of full length SMN from SMN2 in blood can be used as a pharmacodynamic marker but it is important to measure from the baseline of a particular patient i.e. increase relative to baseline.

5) SMN levels can be measured in CSF using a sensitive ELISA.

6) Presence of a reasonable CMAP can be used as a predictive biomarker in that presence of a reasonable CMAP prior to therapeutic intervention predicts maximum effect.

7) In pig models and mice the SMN inducing therapies protect the motor neurons remaining but loss of motor neurons has occurred as the disease progresses.