Disclosures Arthur Burghes

SAB AveXis

Performed studies funded by AveXis

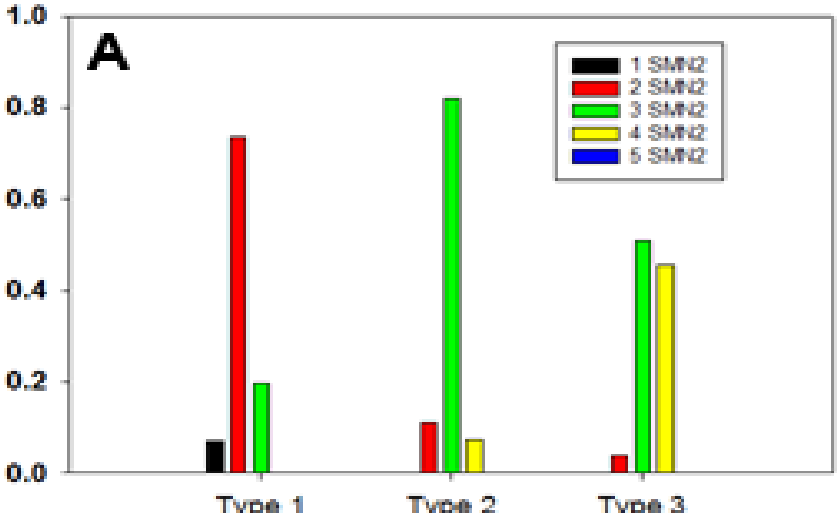
Consulted for Novartis

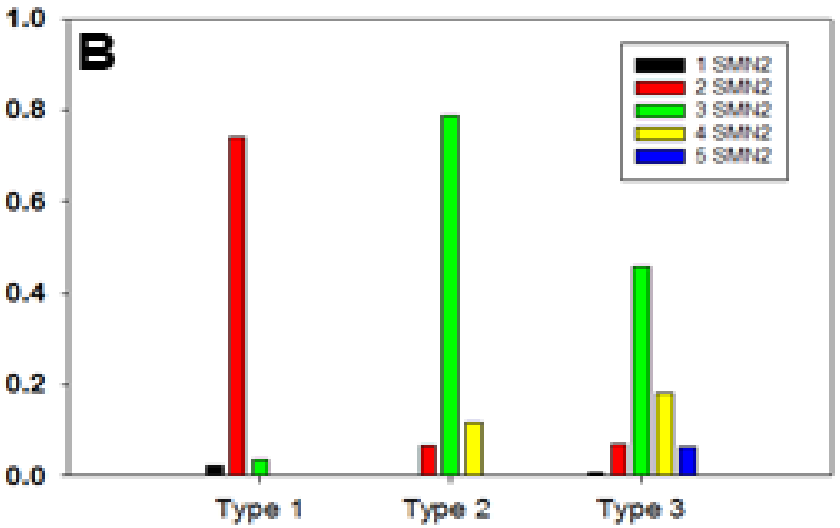
Type of Biomarker	Definition	Actual or Potential Examples in SMA	Validation Dependent Upon Effective Therapy?
Prognostic	Predicts a future clinical outcome	SMN2 copy number → Disease severity	No
Disease Progression	Identifies the severity of disease impact	Compound muscle action potential amplitude →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

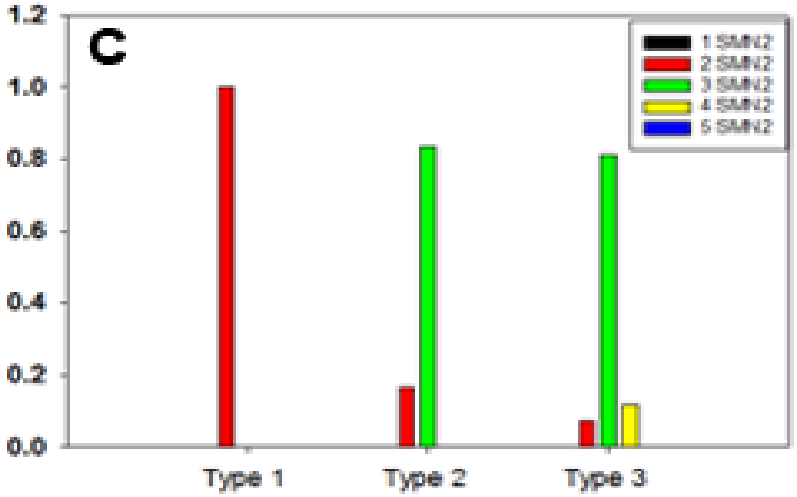
Table 1: Types of Biomarkers. Compound Muscle Action Potential (CMAP), Motor Unit Number Estimation (MUNE), SMA-MAP panel of biomarkers developed by the SMA-Foundation which correlate with the Hammersmith motor function scale

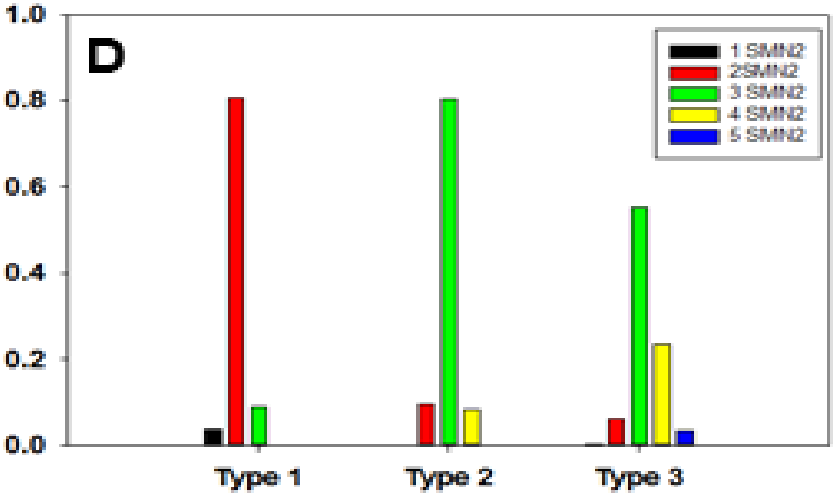
Arnold et al. Development and Testing of Biomarkers in SMA. *in Spinal Muscular Atrophy editors Sumner, C.J., Pauskin, S. Ko, C-P, Academic Press Nov* 15 (2016)

Prognostic Biomarker *SMN2* copy number









A.German population Feldkotter, et al Am J Hum Genet 70, 358-368 (2002).

B. American population Swoboda and Prior

C. Spanish population Bernal, S. et al.. J Med Genet 47, 640-642, 2010).

D. Combined data

Points on SMN2 prognostic

1) SMN2 copy number exceptions between type 1 (2 copies of SMN2) and Type 2 (3copies 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 functio

Kobayashi, et. al. PLoS One, 2012

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

Multi-Analyte Panel Summary

Neuro Next Data

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

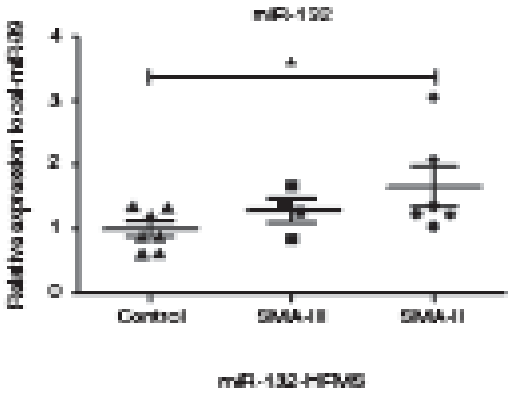
IGFBP6
Myoglobin
Osteopontin
Peptidase D
Chitinase-3-like Protein 1

(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

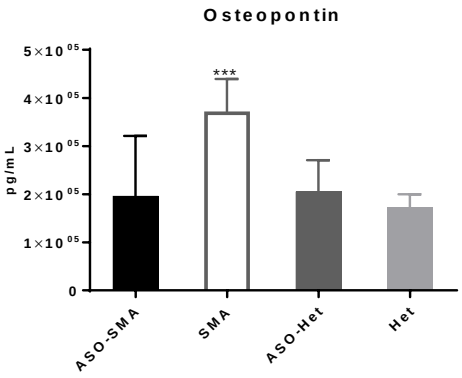
(Catapano et al. Mol Ther Nucleic Acids. 2016 5(7):e331.)

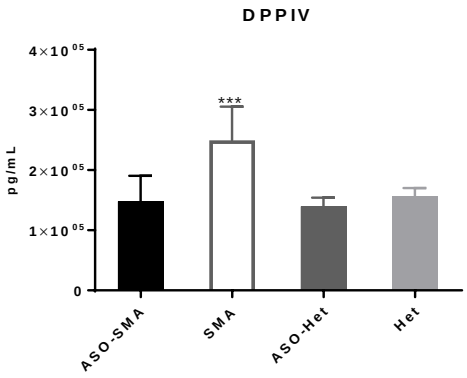
But which if any of these markers responds to therapy?

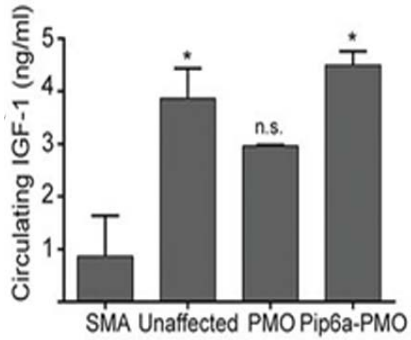
Only have data in mice for change with therapy.

Wexner Medical Center

SMA-MAP and Biomarkers panel analytes that can be tested in mice and are altered in SMA mice

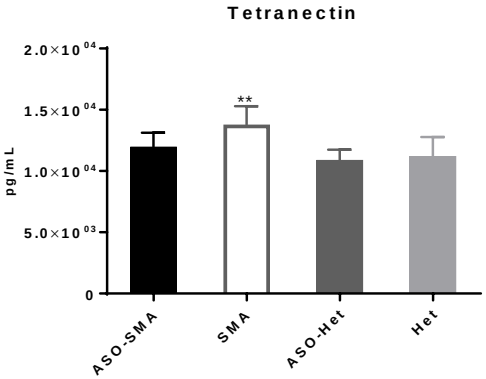


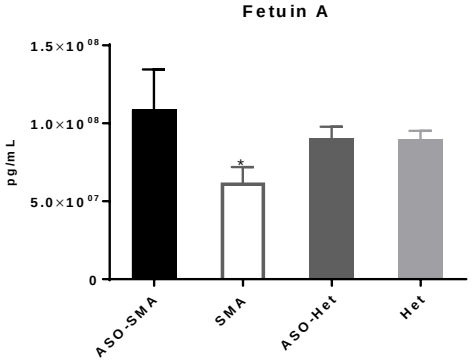


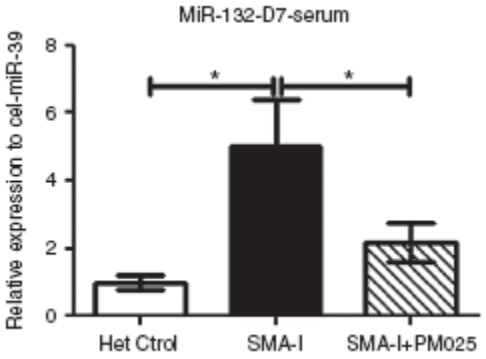


IGF levels at PND7 in treated and untreated Taiwanese SMA mice

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



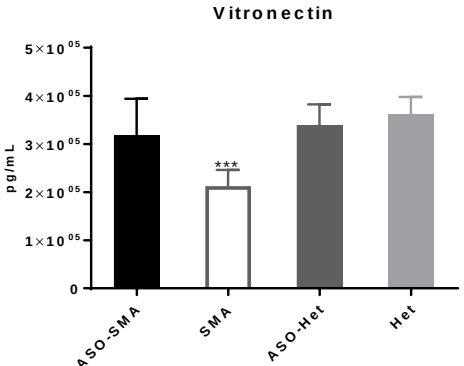




SMA-MAP Serum markers altered at P12 in delta 7 SMA mice and responsive to ASO therapy delivered at P0. 5 out of ten markers tested Responded.

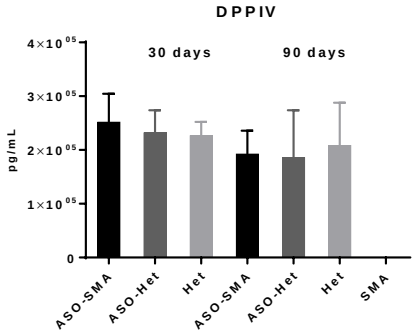
miR132 response to treatment at P0 measured at P2.

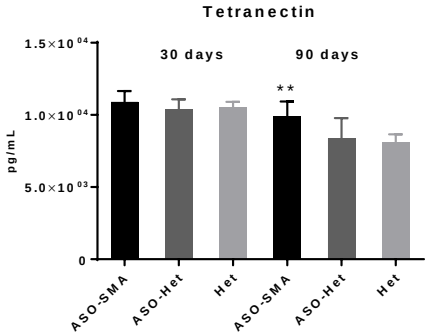
Catapano et al.
Mol Ther Nucleic Acids.
2016 5(7):e331

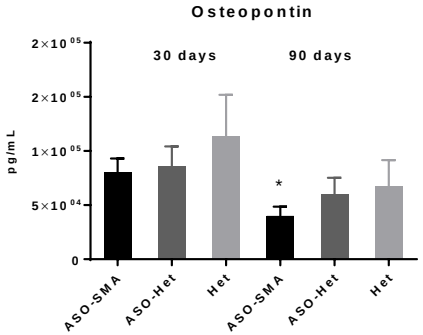


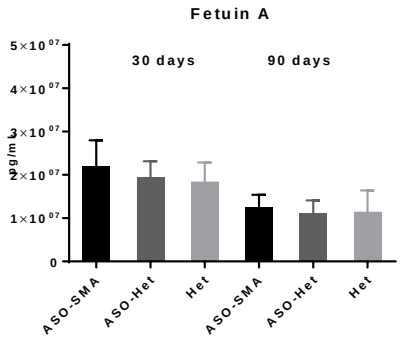
Arnold et al Plos one in Press

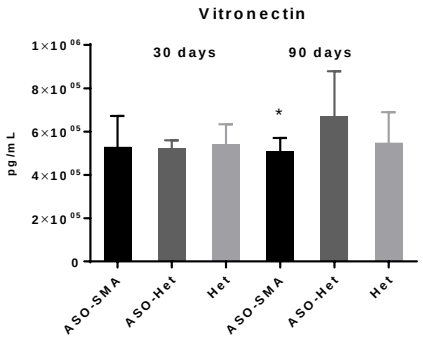
Biomarker response over time



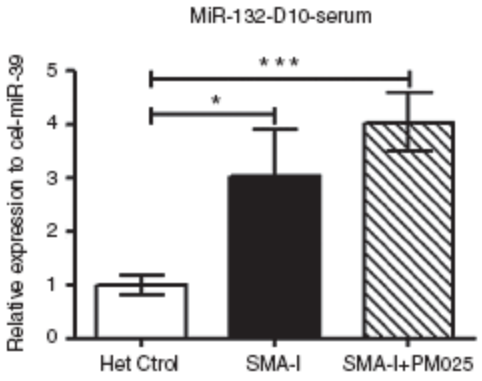








Longitudinal Measures of SMN-corrected biomarkers. Delta 7 SMA mice treated at P0 with ASO and then the biomarker followed with blood samples. DPPIV and fetuin A showed no statistically significant change compared to ASO-Het and Het mice at either P30 or P90. The other 3 analytes, tetranectin, osteopontin,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



miR132 response in Taiwanese SMA mice is lost at P10. Catapano et al. Mol Ther Nucleic Acids. 2016 5(7):e331

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

Biomarker	Change in SMA mice	Normalized with SMN	Correlation with MHFMS (Kobayashi et al. 2013)	Change in SMA (<6 months) (Kolb et al. 2015)
Changed in SMA mice compared with control mice and normalized with SMN				
Osteopontin	↑	Yes	Direct	No Δ
DPPIV	↑	Yes	Direct	↑
Tetranectin	↑	Yes	Direct	↑
Fetuin A	↓	Yes	Inverse	No Δ
Vitronectin	↓	Yes	Inverse	Not performed

Arnold et al Plos One2016 in press

Pharmacodynamic Markers

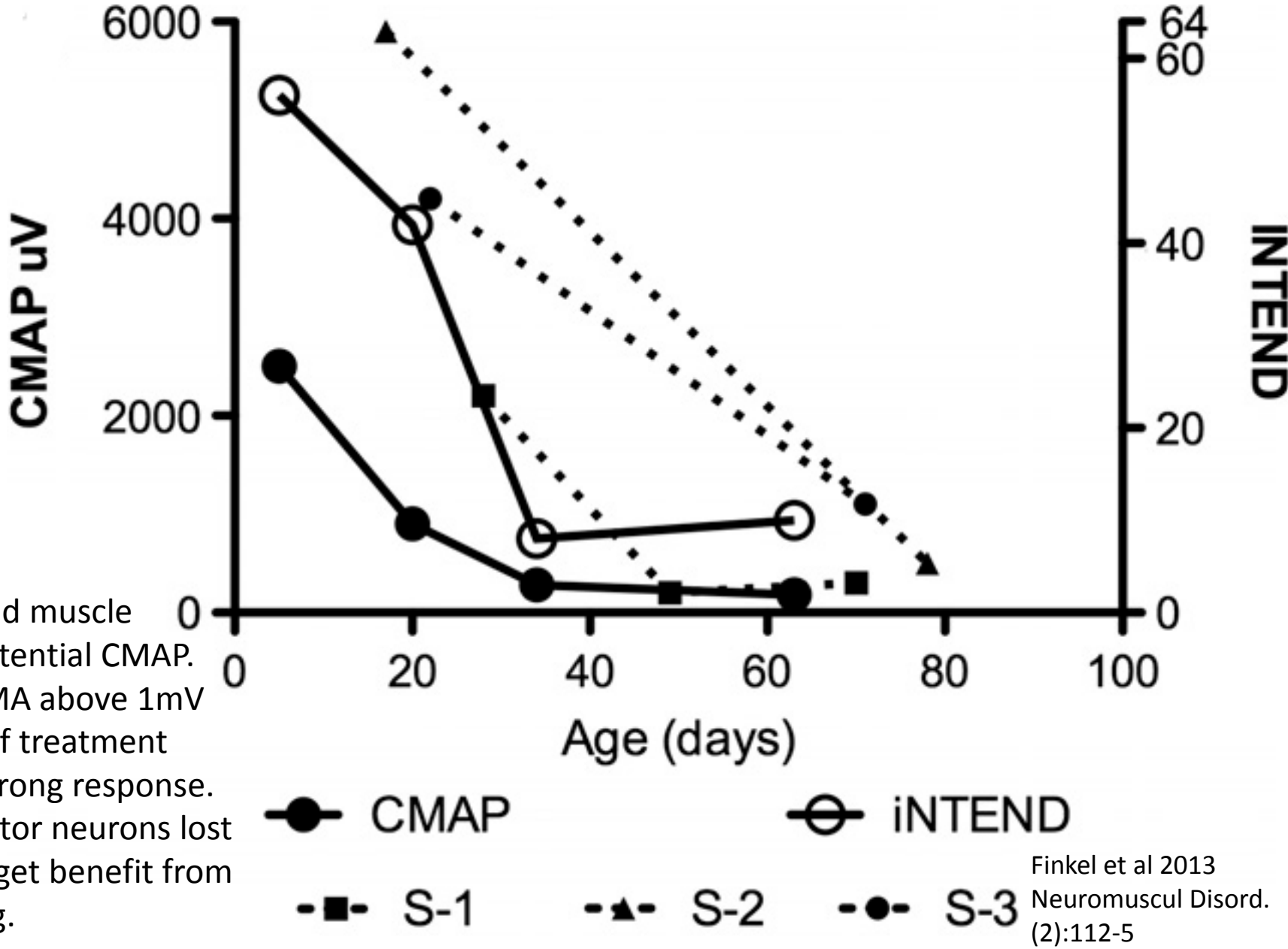
1) Both SMN protein and full length SMN levels should a wide variation of levels in blood and do not correlate with muscle function score or SMN2 copy number. Not a good prognostic marker.

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 ± 0.18 pg/mL; after treatment the levels were 0.59 ± 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)

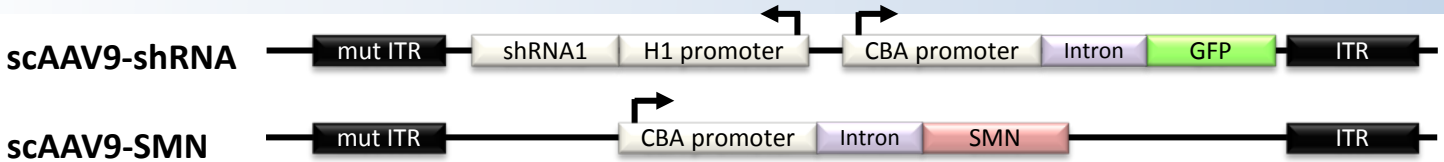
Disease Progression and Predictive

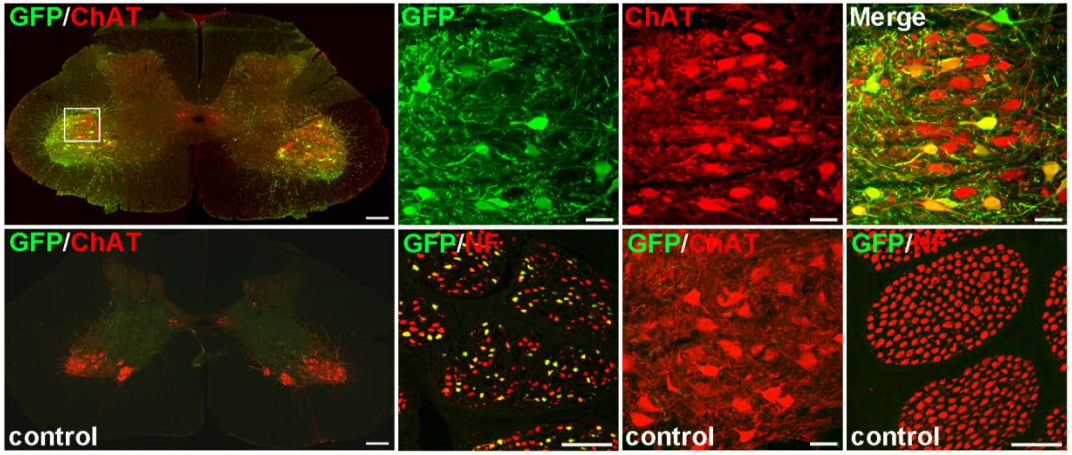


Compound muscle Action Potential CMAP. Type 1 SMA above 1mV on start of treatment predict strong response. Once motor neurons lost Can only get benefit from remaining.

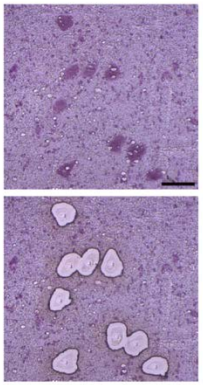
Finkel et al 2013 Neuromuscul Disord. (2):112-5

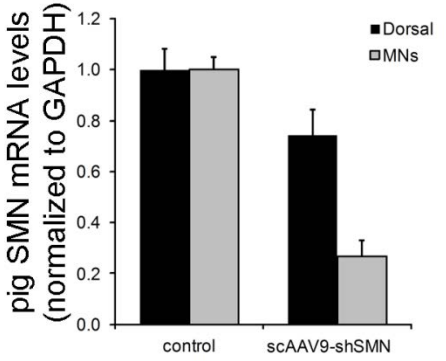
Intrathecal injection of scAAV9-shRNA in 5 day old piglets to create large animal model of SMA what does it predict



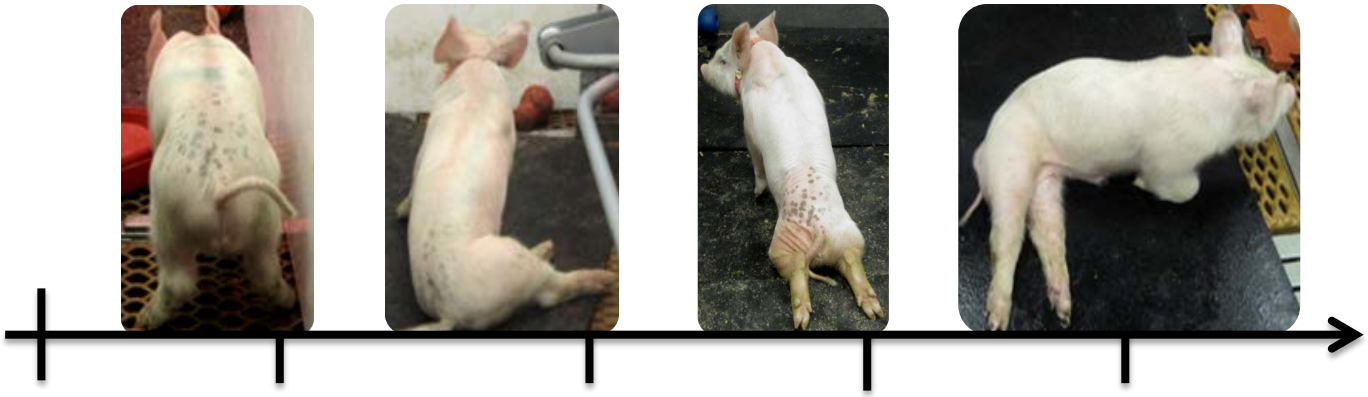


Smn mRNA levels from laser captured motor neurons





Time course of symptoms progression



PND5 injection

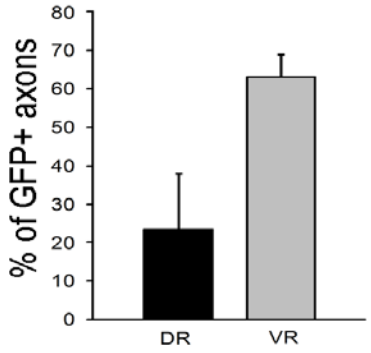
PND24-34 1st sign of weakness Splayed gait

PND33-47 Mainly sitting

PND38-55 Mainly crawling and sitting

PND46-69 sacrifice

Axons labelled



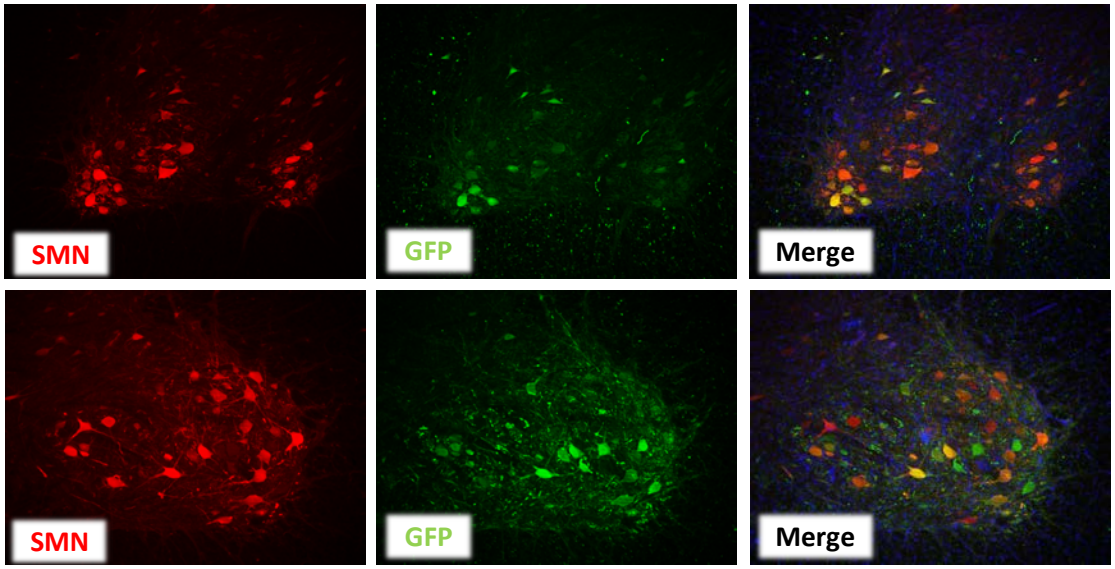
Duque et al Ann Neurol. 2015 77(3):399-414

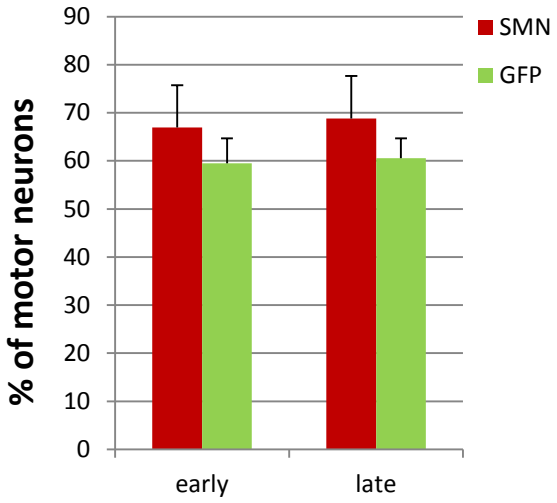
Transduction profile in scAAV9-SMN treated piglets

Group	number of animal	Age at sacrifice	Immunosuppressive treatment	Age at injection		Dose vector (vg/kg)	
				scAAV9-shRNA	scAAV9-SMN	scAAV9-shRNA	scAAV9-SMN
Control	6	79 ± 3	-	-	-	-	-
shRNA	5	61 ± 7	Prograf	PND5	-	$6.5x10^{12}$	-
SMN early	5	71 ± 2	Prograf + Cellcept	PND5	PND6	$6.5x10^{12}$	$8x10^{12}$
SMN late	5	73 ± 3	Prograf + Cellcept	PND5	PND33-36	$6.5x10^{12}$	$8x10^{12}$

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

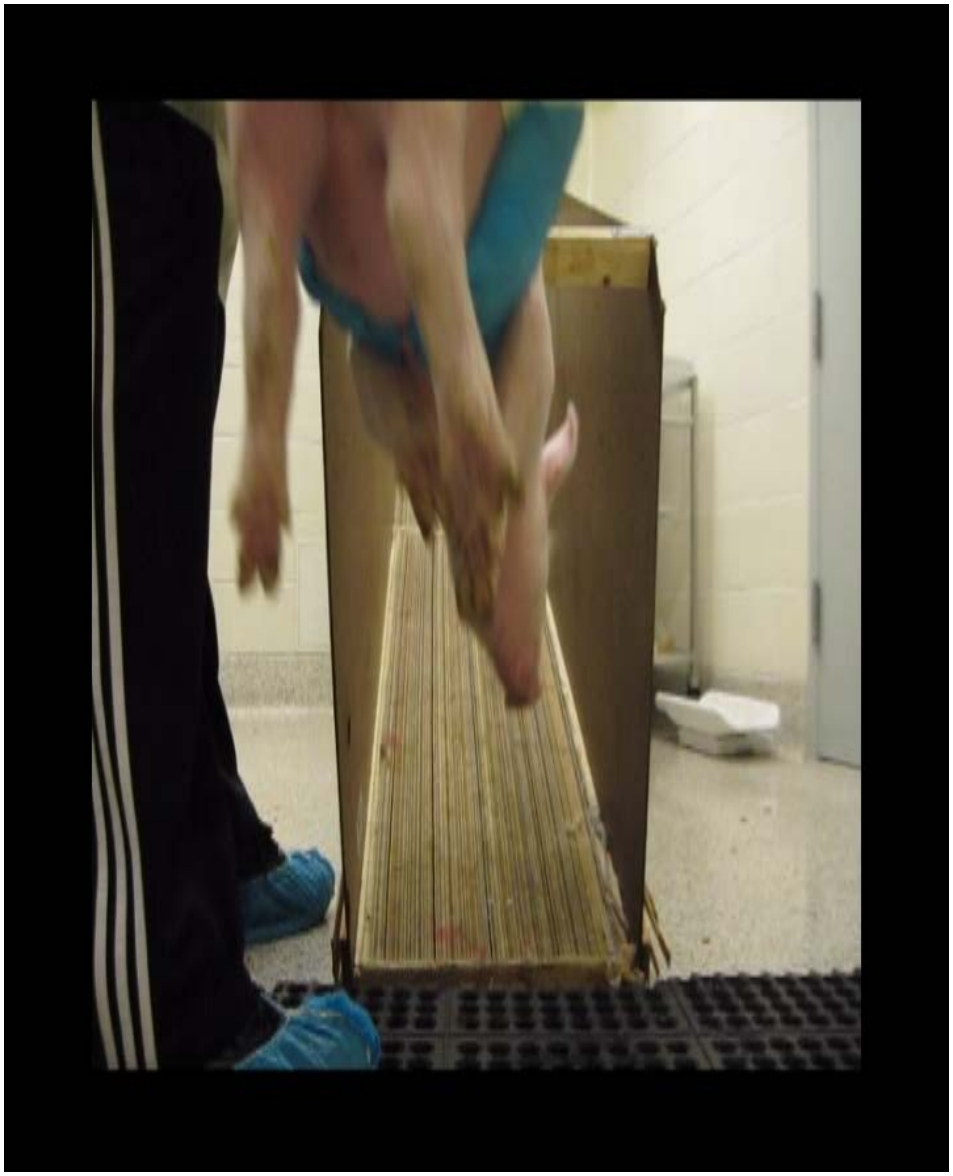
Spinal cord section from lumbar 6 segment stained for GFP and human SMN (human specific antibody)





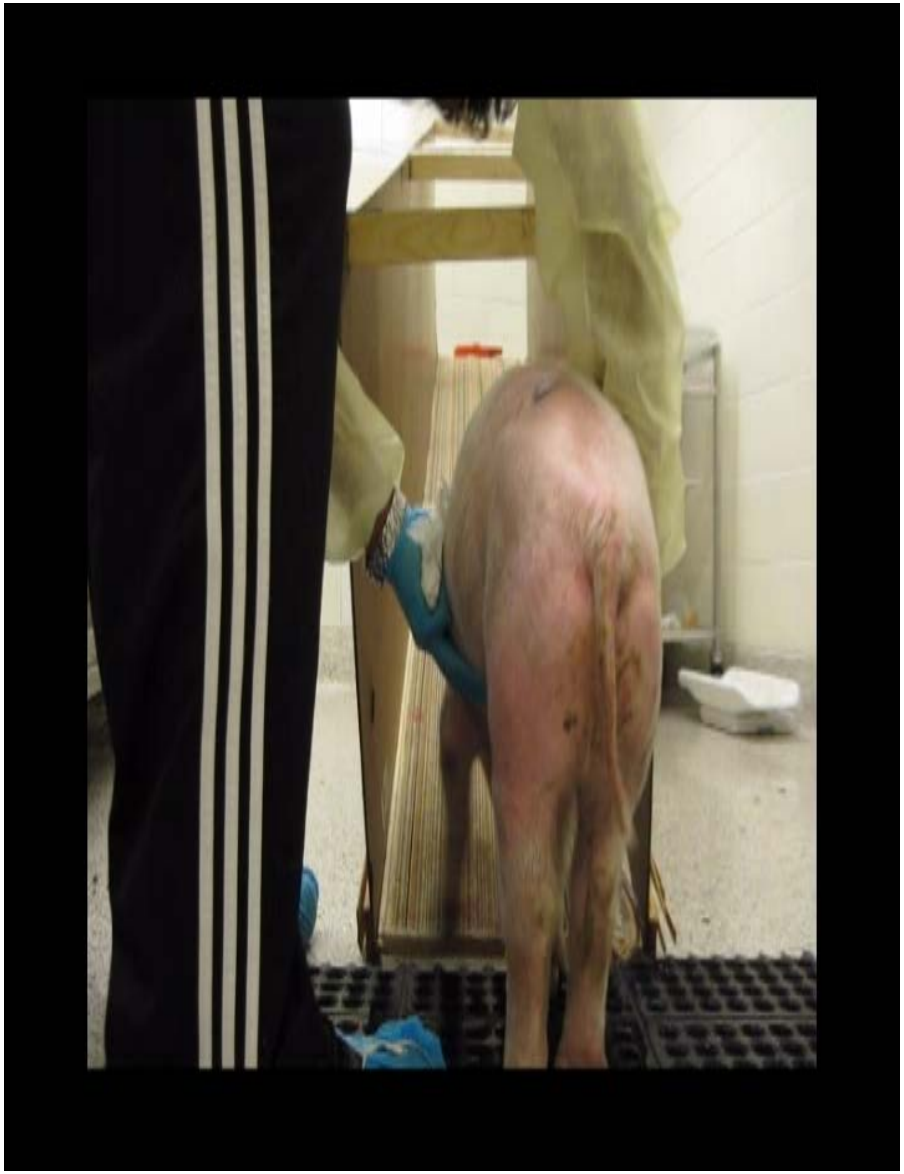
Duque et al Ann Neurol. 2015 Mar;77(3):399-414

Control



Early rescue

Late rescue

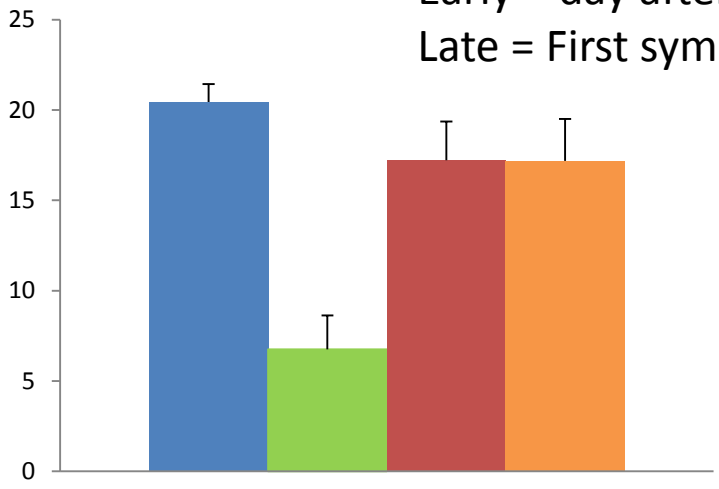


No rescue

Correlation of MUNE, CMAP and Motor Neuron loss with SMN restoration in the pig.

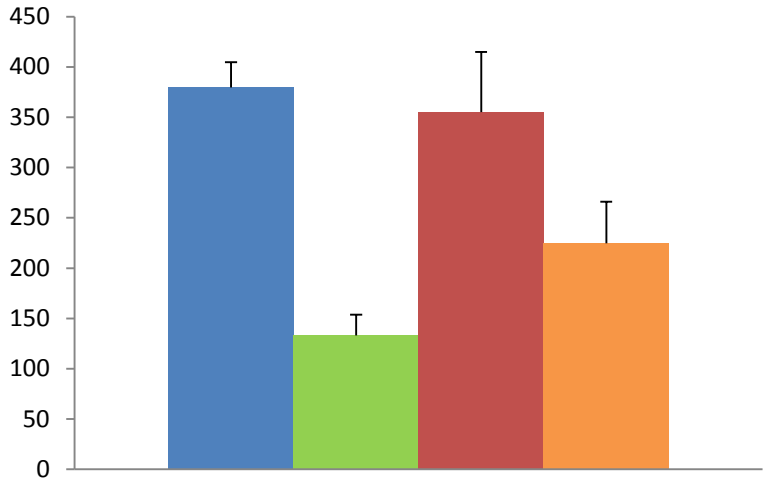
CMAP

Early = day after shRNA
Late = First symptoms



Motor neuron path also improved

MUNE



Group	Fibrillations	Percentage
shRNA (5pigs)	5	100%
Control (6pigs)	0	0%

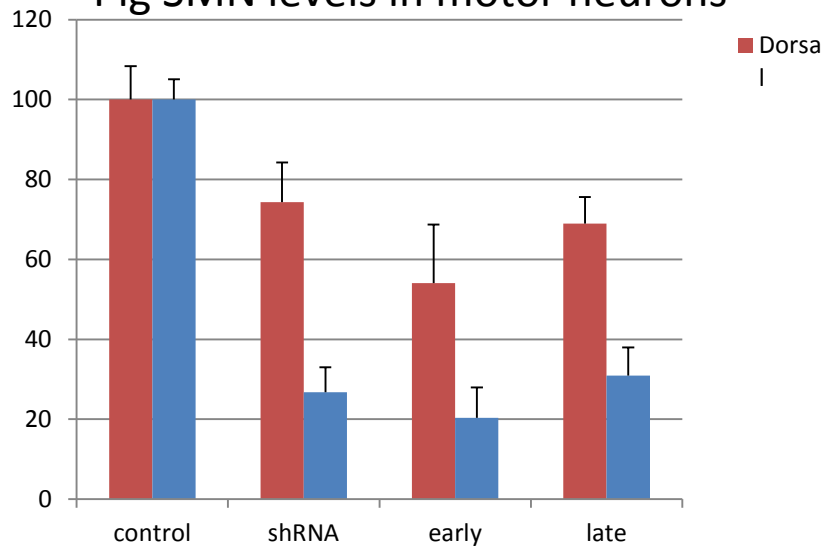
Normal

shRNA

Early rescue

Late rescue

Pig SMN levels in motor neurons



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 oh 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