

SUPPLEMENTARY MATERIAL

Table of content

Statistical methodology	Pag 2
Supplementary Figure 1	Pag 4
Supplementary Figure 2	Pag 6
Supplementary Figure 3	Pag 8
Supplementary Table 1	Pag 9
Supplementary Table 2	Pag 10
Supplementary Table 3	Pag 11
Supplementary Table 4	Pag 12

Statistical methodology

Mixed model analysis

A linear mixed model (estimated using REML and nlptwrap optimizer) was fitted to predict score with time, months at OA infusion (cat), Sex, pretreatment with other pharmacological treatment, weight at OA infusion (cat), SMA 1 severity, CHOP INTEND score at baseline and SMN2 copy number (formula: $\text{score} \sim \text{time} * \text{months at OA infusion} + \text{Sex} + \text{pretreatment with other pharmacological treatment} + \text{weight at OA infusion (cat)} + \text{SMA 1 severity} + \text{CHOP INTEND score at baseline} + \text{SMN2 copy number}$). The model included the patients as random effect (formula: $\sim 1 | \text{ID}$). A complete model re-fit with a standardized version of data was performed, hence standardizing variables before fitting the model (Neter, Wasserman, and Kutner 1989). 95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation. The analysis was performed with R package “lme4” and “lmerTest”.

Negative binomial regression

A negative-binomial model (estimated using ML) was fitted to predict how many AST peaks with Sex, SMA 1 severity, months at OA infusion (cat), pretreatment with other pharmacological treatment, weight at OA infusion (cat) and $\log(\text{time})$ (formula: $\text{AST peaks} \sim \text{Sex} + \text{SMA 1 severity} + \text{months at OA infusion (cat)} + \text{pretreatment with other pharmacological treatment} + \text{weight at OA infusion (cat)} + \text{offset}(\log_time)$). A complete model re-fit with a standardized version of data was performed, hence standardizing variables before fitting the model (Neter, Wasserman, and Kutner 1989). 95% Confidence Intervals (CIs) and p-values were computed using a Wald z-distribution approximation. The analysis was performed with R package “MASS”.

Cox regression model analyses

The Hazard Ratio and its 95% confidence interval were calculated with the Cox regression model (univariable and multivariable). The analysis was performed with R package “survival”.

Univariable model formulas: $\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim \text{SMA_Type}, \text{data} = \text{COXF})$;

$\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim \text{nusinersen_cat}, \text{data} = \text{COXF})$; $\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim$

$\text{age_OA_cat}, \text{data} = \text{COXF})$; $\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim \text{WEIGHT_CAT}, \text{data} = \text{COXF})$;

$\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim \text{SMN2_copy}, \text{data} = \text{COXF})$; $\text{coxph}(\text{Surv}(\text{time_at_fu}, \text{sitting_acquired}) \sim$

$\text{Sex}, \text{data} = \text{COXF})$

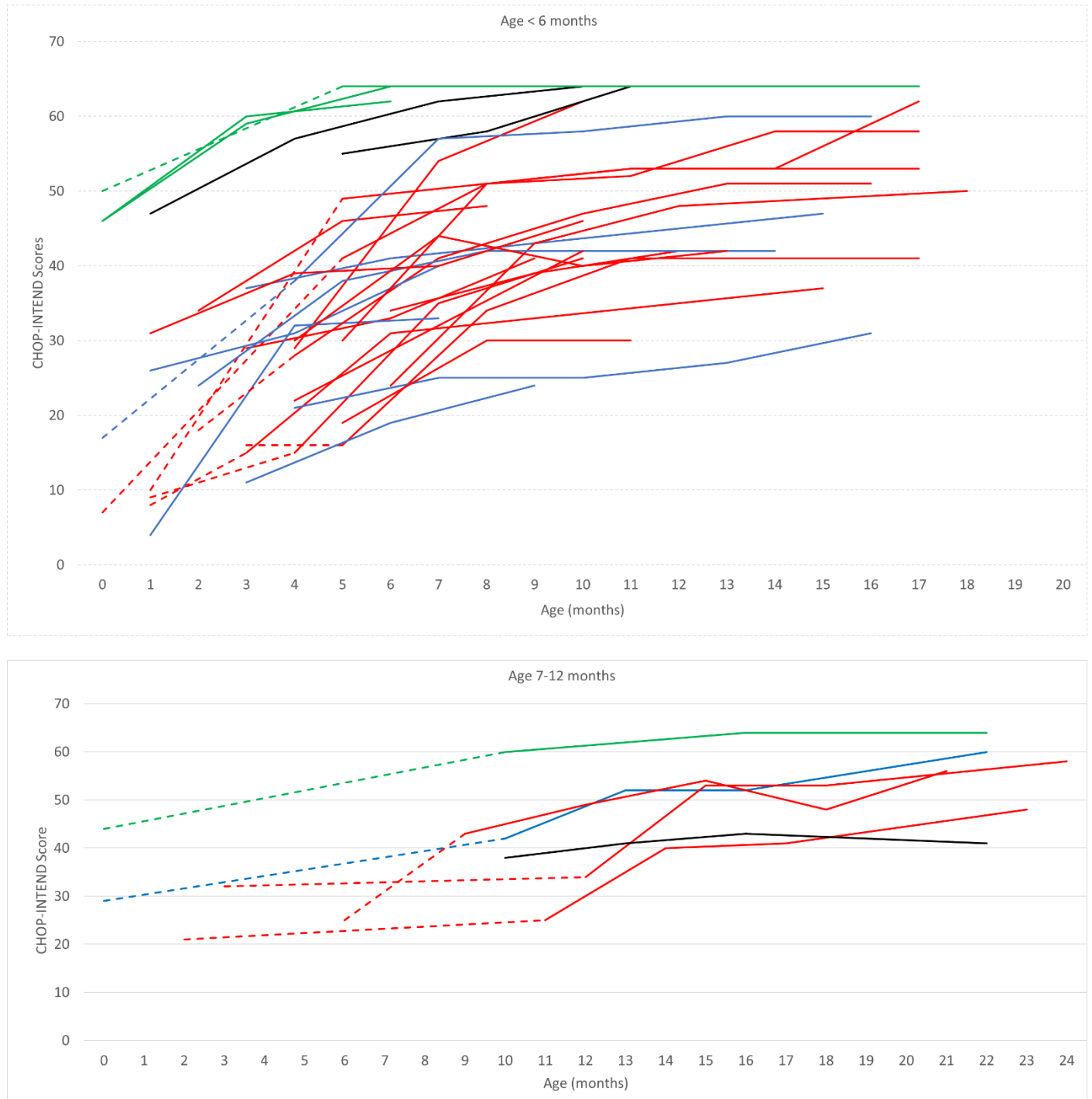
Multivariable model formula:

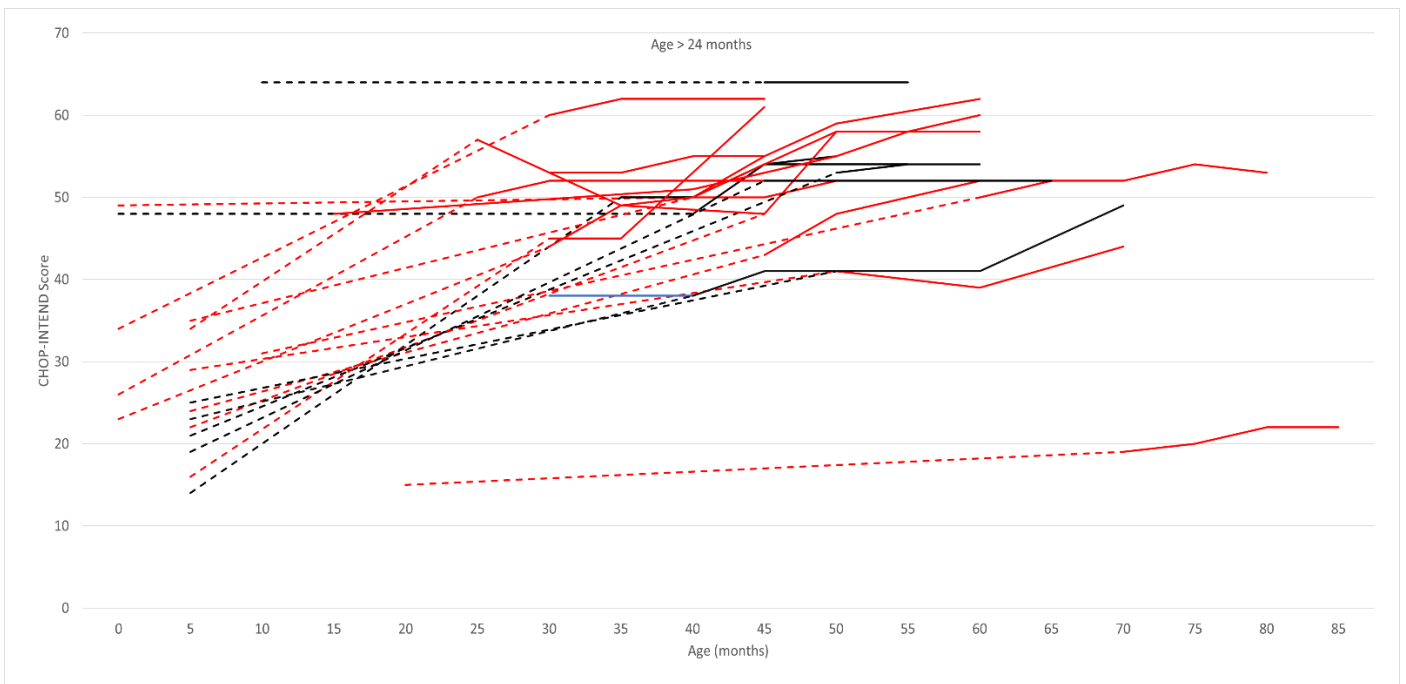
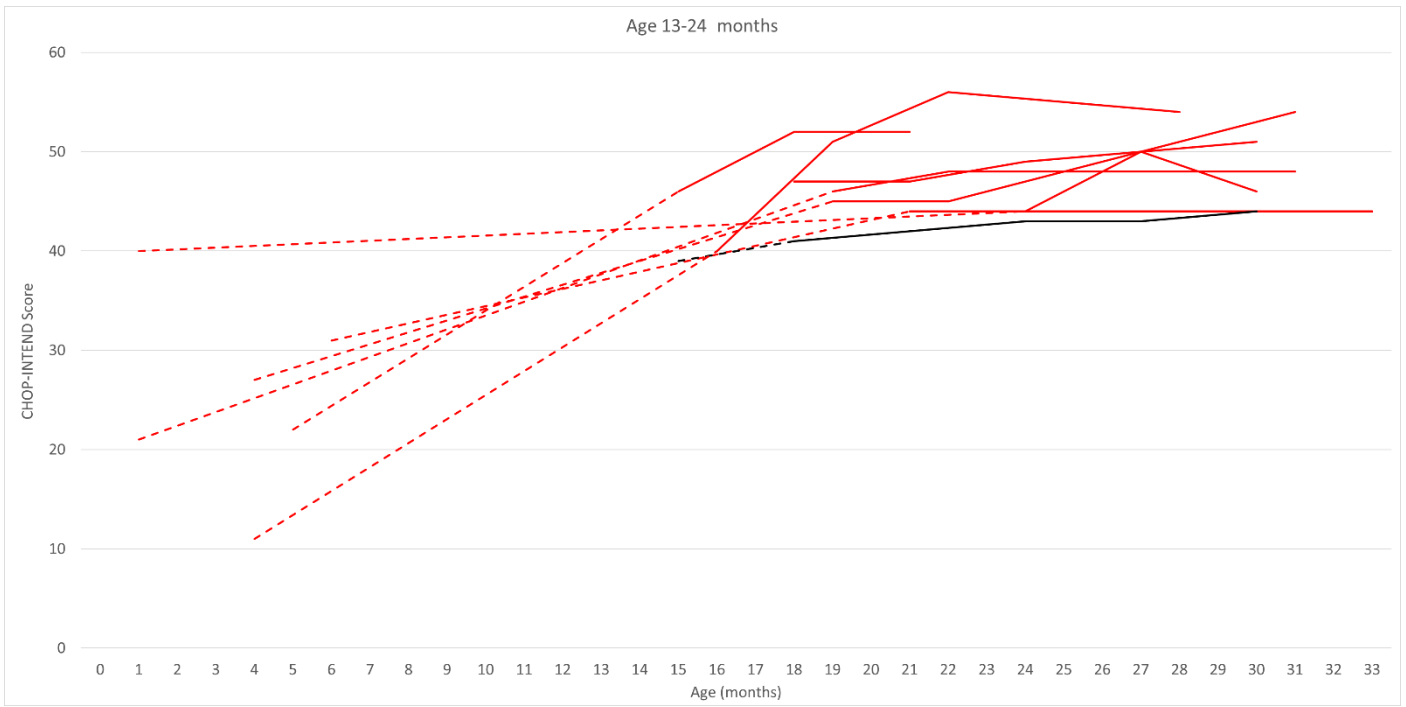
```
coxph(Surv(time_at_fu, sitting_acquired) ~ SMA_Type + age_OA_cat + nusinersen_cat + WEIGHT_CAT + Sex +  
SMN2_copy , data = COXF)
```

Supplementary Figure 1

Individual trajectories of CHOP INTEND score over time in patients subdivided according to age at treatment, treatment status and severity.

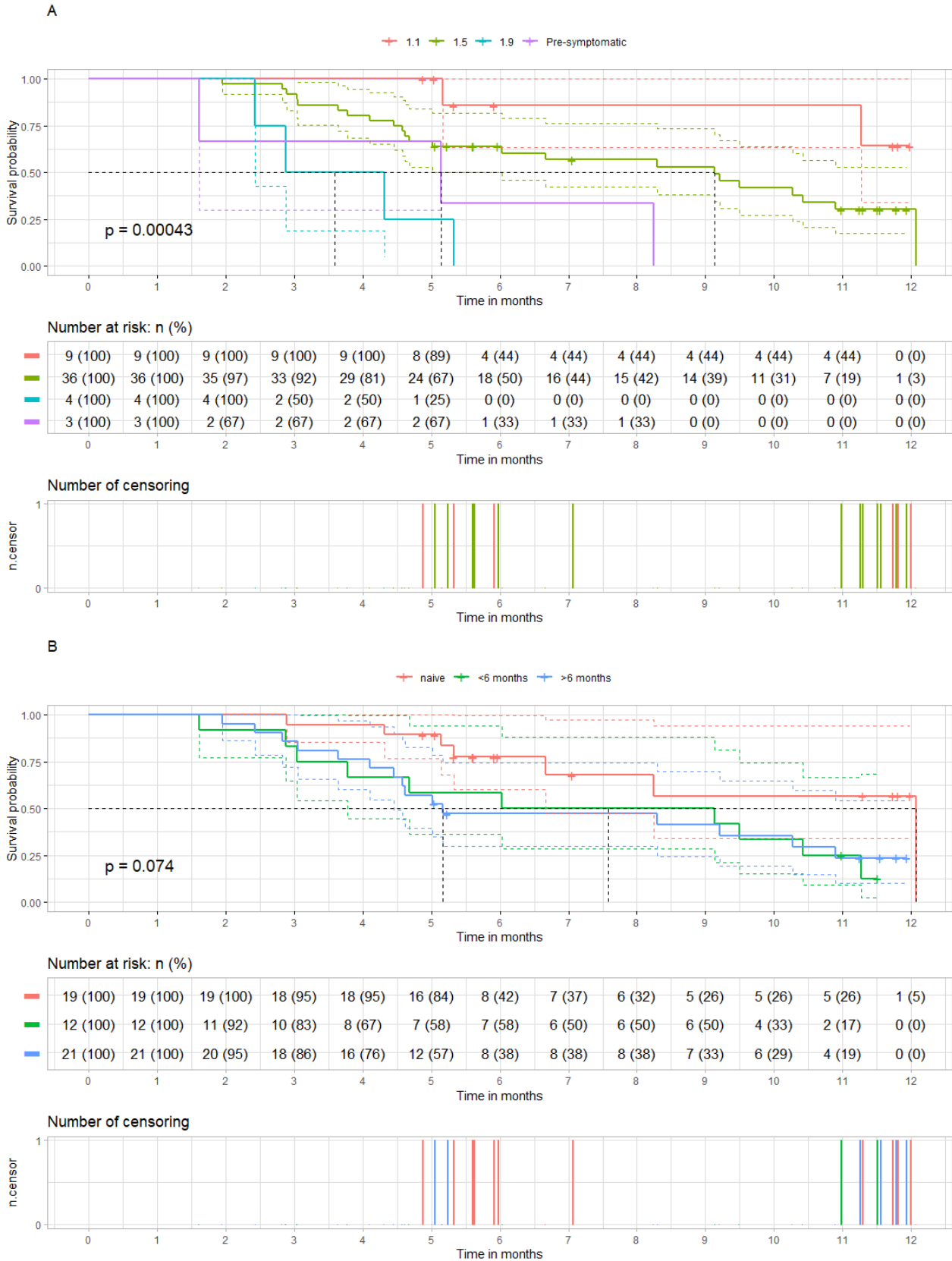
Legend: dashed-line: pre-treatment with nusinersen/risdiplam; continuous line: treatment with OA; green line: pre-symptomatic; blu line: 1·1; red line: 1·5; black line: 1·9.



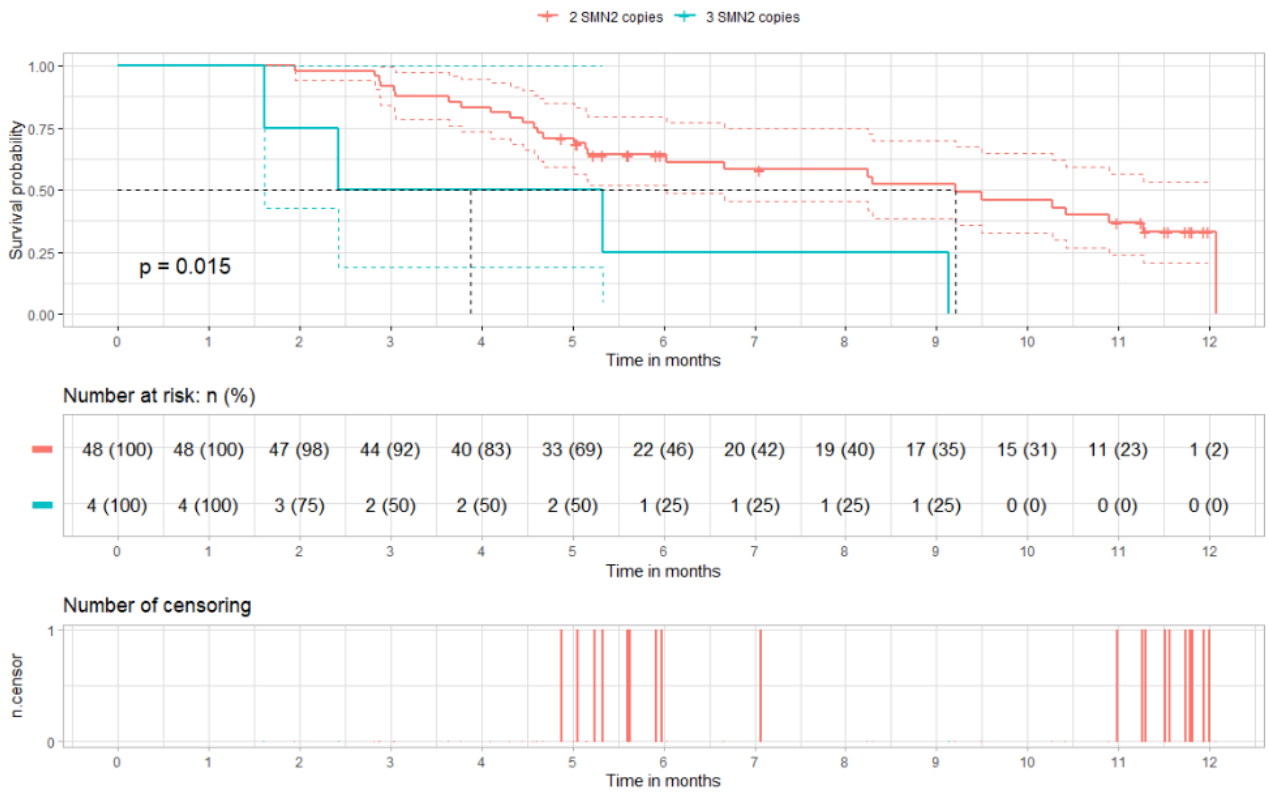


Supplementary Figure 2

Key to figure: Panel A: subdivided by SMA I severity, Panel B: subdivided by age at OA treatment, Panel C: SMN2 copy number

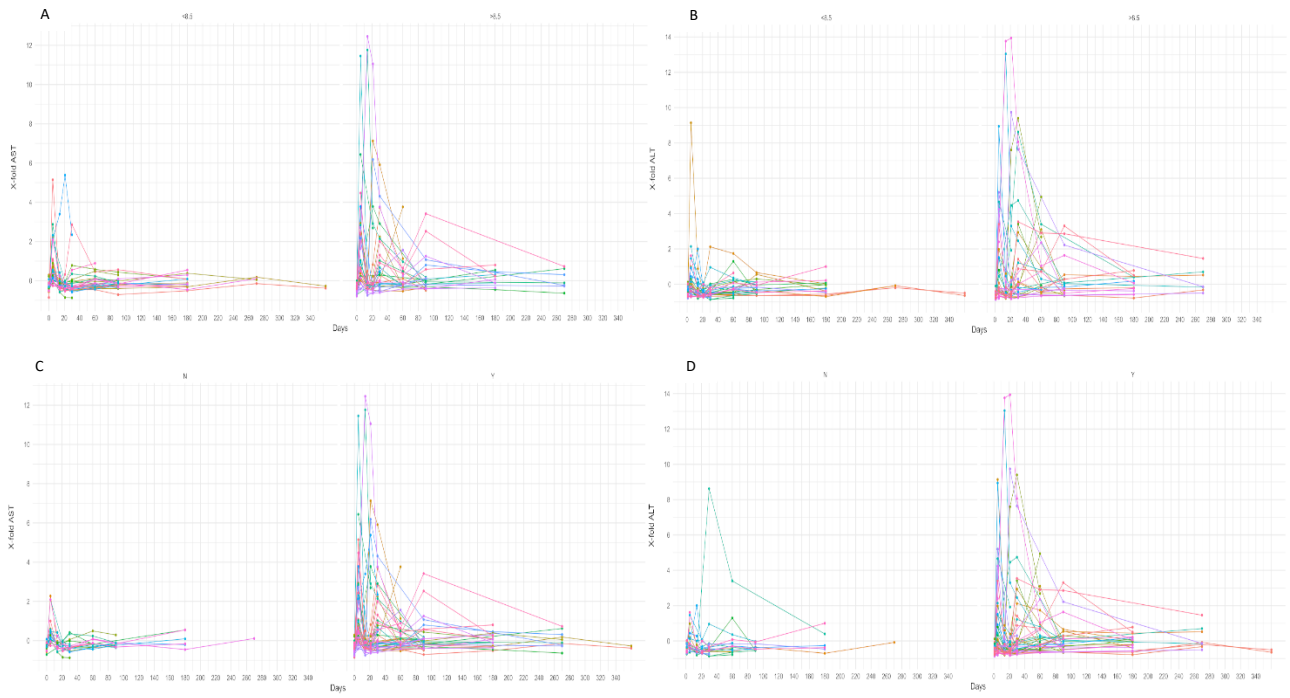


C



Supplementary Figure 3

Individual trajectories of x-fold AST and ALT subdivided by weight (A – B) and treatment (C – D).



Supplementary Table 1

Post-hoc contrast for time*age at treatment.

Time*Age at OA treatment	contrast	estimate	SE	df	lower.CL	upper.CL	t.ratio	p.value
<6 months	T0-T3	-11.333	1.26	129.0	-15.6677	-6.9989	-9.021	<.0001**
	T0-T6	-13.933	1.26	129.0	-18.2677	-9.5989	-11.091	<.0001**
	T3-T6	-2.600	1.26	129.0	-6.9344	1.7344	-2.070	1.0000
	T0-T12	-16.667	1.26	129.0	-21.0011	-12.3323	-13.267	<.0001**
	T3-T12	-5.333	1.26	129.0	-9.6677	-0.9989	-4.245	0.0027**
	T6-T12	-2.733	1.26	129.0	-7.0677	1.6011	-2.176	1.0000
7-24 months	T0-T3	-5.750	1.40	129.0	-10.5960	-0.9040	-4.094	0.0049**
	T0-T6	-7.500	1.40	129.0	-12.3460	-2.6540	-5.340	<.0001**
	T3-T6	-1.750	1.40	129.0	-6.5960	3.0960	-1.246	1.0000
	T0-T12	-9.750	1.40	129.0	-14.5960	-4.9040	-6.942	<.0001**
	T3-T12	-4.000	1.40	129.0	-8.8460	0.8460	-2.848	0.3382
	T6-T12	-2.250	1.40	129.0	-7.0960	2.5960	-1.602	1.0000
>24 months	T0-T3	-2.632	1.12	129.0	-6.4828	1.2196	-2.358	1.0000
	T0-T6	-3.842	1.12	129.0	-7.6933	0.0091	-3.442	0.0514
	T3-T6	-1.211	1.12	129.0	-5.0617	2.6407	-1.084	1.0000
	T0-T12	-5.105	1.12	129.0	-8.9565	-1.2541	-4.574	0.0007**
	T3-T12	-2.474	1.12	129.0	-6.3249	1.3775	-2.216	1.0000
	T6-T12	-1.263	1.12	129.0	-5.1144	2.5880	-1.132	1.0000

Supplementary Table 2

Differences between stratification subgroups.

	Stratification subgroups	N	Median (IQR)	Mean (SD)	P value
					Kruskal wallis
Age at OA treatment	<i><6mo</i>	14	9 (4-12)	10.0 (3.48)	Chi-square: 25.605, p<0.001, df=2
	<i>7-24mo</i>	9	20 (8)	20.3 (5)	
	<i>>24mo</i>	9	49 (4)	48.3 (6.56)	
Sex	<i>Female</i>	21	25 (33)	29.5(18)	Chi-square: 6.561, p=0.010, df=1
	<i>Male</i>	11	10 (8.5)	12.7 (6.22)	
Weight	<i><8500</i>	18	10.5 (7.5)	13.8 (10.4)	Chi-square: 16.397, p<0.001, df=1
	<i>>8500</i>	14	39.5 (27)	36.4 (15.2)	
SMN2 copy number	2	28	16.5 (26)	24.2 (16.9)	N/A
	3	4	13 (19.2)	20.2 (19.3)	
SMA 1 severity of the disease	<i>1-1</i>	2	25 (9)	25 (12.7)	N/A
	<i>1-5</i>	23	20 (33.2)	26.5 (17.2)	
	<i>1-9</i>	4	11.5 (15.5)	19.5 (19.3)	
	<i>Presymptomatic</i>	3	7 (1.5)	7.33 (1.53)	
Pretreatment with nusinersen*	<i>Naive</i>	7	9 (6.25)	10.4 (4.29)	Chi-square: 20.036, p<0.001, df=2
	<i><6 months</i>	10	10.5 (7.25)	12.3 (4.95)	
	<i>>6 months</i>	15	45 (24.5)	37.5 (15.0)	

Supplementary Table 3

AST and ALT increase during follow-up period. Key to table: * increase before 14 days and at least one another increase between 15-30 days after OA treatment; ** increase between 14-30 days and at least one another increase after 30 days after OA treatment

		Days from OA treatment				
		14 days	15-30 days	31-90 days	<14days + 14-30days*	14-30 days + >30days**
AST	Total (N)	13	6	2	5	0
	<i>≤6 months</i>	5	0	1	0	0
	<i>7-24 months</i>	6	0	0	2	0
	<i>>24 months</i>	2	6	1	3	0
	Median AST value (range)	189 (170-685)	161.5 (178-395)	228 (194-262)	242 (172-740)	N/A
ALT	Total (N)	6	4	4	3	3
	<i>≤6 months</i>	1	1	1	0	0
	<i>7-24 months</i>	4	0	0	1	1
	<i>>24 months</i>	1	3	3	2	2
	Median ALT value (range)	296.5 (157-507)	209 (156-287)	210 (168-297)	224 (170-747)	430 (161-2509)

Supplementary Table 4

Negative binomial regression analysis adjusting per days of follow-up.

AST				
dispersion ratio = 2.742 Pearson's Chi-Squared = 167.289 p-value = < 0.001				
term	estimate	conf.low	conf.high	p.value
(Intercept)	0.00	0.00	0.02	<0.001
Sex_M	0.68	0.21	2.16	0.46
Disease severity 1.5	1.49	0.26	11.94	0.659
Disease severity 1.9	0.36	0.04	4.15	0.377
Disease severity Presymptomatic	1.48	0.13	19.08	0.74
Age_class7-24	7.52	1.72	37.60	0.007*
Age_class>24	5.76	1.03	36.41	0.045*
Pretreatment_with_Nusinersen_Y	1.64	0.28	10.92	0.56
Weight_kg	0.90	0.71	1.16	0.43
ALT				
dispersion ratio = 1.352 Pearson's Chi-Squared = 78.412 p-value = 0.038				
term	estimate	conf.low	conf.high	p.value
(Intercept)	0.00	0.00	0.00	<0.001
Sex_M	0.72	0.26	2.13	0.51
Disease severity 1.5	1.36	0.19	28.01	0.79
Disease severity 1.9	0.53	0.05	12.39	0.62
Disease severity Presymptomatic	4.76	0.42	117.69	0.24
Age_class7-24	9.07	1.67	72.26	0.02*
Age_class>24	11.08	1.51	123.60	0.03*
Pretreatment_with_Nusinersen_Y	0.48	0.07	3.11	0.45
Weight_kg	1.11	0.90	1.41	0.32