Prognostic Factors and Treatment-Effect Modifiers in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a rare, progressive neuromuscular disease characterized by loss of motor neurons and muscle atrophy. Untreated infants with type 1 SMA do not achieve major motor milestones, and death from respiratory failure typically occurs before 2 years of age. Individuals with types 2 and 3 SMA exhibit milder phenotypes and have better functional and survival outcomes. Herein, a systematic literature review was conducted to identify factors that influence the prognosis of types 1, 2, and 3 SMA. In untreated infants with type 1 SMA, absence of symptoms at birth, a later symptom onset, and a higher survival of motor neuron 2 (*SMN2*) copy number are all associated with increased survival. Disease duration, age at treatment initiation, and, to a lesser extent, baseline function were identified as potential treatment-modifying factors for survival, emphasizing that early treatment with disease-modifying therapies (DMT) is essential in type 1 SMA. In patients with types 2 and 3 SMA, factors considered prognostic of changes in motor function were *SMN2* copy number, age, and ambulatory status. Individuals aged 6–15 years were particularly vulnerable to developing complications (scoliosis and progressive joint contractures) which negatively influence functional outcomes and may also affect the therapeutic response in patients. Age at the time of treatment initiation emerged as a treatment-effect modifier on the outcome of DMTs. Factors identified in this review should be considered prior to designing or analyzing studies in an SMA population, conducting population matching, or summarizing results from different studies on the treatments for SMA.

Spinal muscular atrophy (SMA) is a rare, progressive, genetic neuromuscular disease, characterized by loss of motor neurons, muscle atrophy, and weakness.[1] SMA is caused by insufficient levels of the survival of motor neuron (SMN) protein due to deletion and/or mutations in the *SMN1* gene located on chromosome 5q.[2–4] A second, paralogous gene *SMN2* also encodes SMN protein but, due to alternative splicing of *SMN2* messenger RNA, only produces low levels of functional SMN protein that are insufficient to fully compensate for the lack of *SMN1*.[5] *SMN2* copy number is polymorphic in the population and higher copy numbers are reported to be inversely correlated with disease severity.[6,7] However, this correlation is not absolute, as protective *SMN2* mutations and polymorphisms in other genes that could modify the disease course have also been described.[8]

SMA encompasses a broad spectrum of disease,[9] classified into five clinical types: types 0, 1, 2, 3, and 4 SMA,[10] defined by age of onset and highest motor milestone achieved, based on natural history.[11,12] Type 0 is rare and is the most severe form of SMA with a prenatal or neonatal onset.[13] Type 1 SMA is the most common form, affecting roughly 58% of all individuals with SMA, while ~ 29% and 13% of individuals with SMA are of the type 2 or type 3 phenotype, respectively.[14] Type 4, (adult-onset SMA), is also rare (representing <5% of cases) and is the mildest form of the disease.[10] Due to the rarity of type 0 and type 4 within the SMA population, this review will focus on types 1, 2, and 3 SMA.

Infants with type 1 SMA frequently possess only two copies of *SMN2*.[6,15] Type 1 SMA is characterized by hypotonia and severe muscle weakness that becomes evident in the first 6 months of life.[11,16,17] Untreated infants with type 1 SMA never achieve major motor milestones, have poor, if any, head control, and never sit independently.[11,16,17] Swallowing is also compromised, with more than half of infants requiring feeding support at 8 months of age.[18] Natural history studies have described a severely shortened lifespan for patients with two *SMN2* copies,[18,19] with 68% of infants dying before the age of 2 years, and 82% before the age of 4 years.[12,20]

Individuals with type 2 SMA generally have three copies of *SMN2*.[21–23] Symptom onset typically occurs between 6 and 18 months of age. Individuals can sit unsupported, and some can stand with braces, but they will never achieve independent ambulation.[24,25] Type 2 SMA can be further stratified into two subgroups based on age at independent sitting: types 2a (>8 months of age) and 2b (≤8 months).[26] Disease progression can vary greatly;

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however, nearly all individuals will develop scoliosis due to weakness of the axial muscles.[27] Untreated children at the severe end of the spectrum often die in childhood or adolescence, while stronger patients survive into early adulthood.[12,28] Respiratory muscle weakness, affecting disproportionately the intercostal muscles, and poor bulbar function have traditionally been the common causes of death among patients with type 2 SMA.[29]

Individuals with type 3 SMA typically have three or four copies of the *SMN2* gene, resulting in a milder disease course and normal life expectancy.[27] Type 3 SMA is further categorized into two subgroups by age at onset of symptoms: types 3a (< 3 years) and 3b (≥ 3 years).[30] There is broad heterogeneity in the symptoms exhibited: Some individuals will lose the ability to walk (most of the 3a patients) before adulthood, while others may experience only minor muscle weakness.[31] Scoliosis is prevalent in individuals with type 3a SMA who lose the ability to walk in childhood, but it is less common in type 3b.[27]

Improvements in standard of care (SoC) guidelines[31-33] and advances in respiratory and nutritional support have led to increased survival in patients with type 1 and type 2 SMA.[34] Additionally, with the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals of nusinersen (Spinraza),[35,36] onasemnogene abeparvovec-xioi (Zolgensma)[37,38] and risdiplam (Evrysdi),[39,40] the pattern of disease progression in SMA is changing.[34] As patients live longer, the natural history of SMA and the impact of these emerging treatments needs further examination.

There is a gap in the literature regarding the synthesis of studies which evaluate factors that are prognostic of the natural history of SMA or are predictive of the efficacy of current treatments. Therefore, we conducted a systematic literature review (SLR) of published clinical trials and observational studies in order to identify potential treatment-effect modifiers (factors that affect the efficacy of a given treatment) and prognostic factors (factors that affect the natural course of the disease) in patients with SMA.

Methods

Study identification

An SLR of randomized controlled trials (RCTs) and observational studies in types 1, 2, and 3 SMA was performed. Searches were conducted in Embase (Amsterdam, Netherlands) and MEDLINE databases using the embase.com interface from January 1, 2000 to April 30, 2019. Supplementary searches included a bibliographic search to identify key studies not retrieved from the structured searches and a health technology assessment documentation search to identify any relevant data for nusinersen and onasemnogene abeparvovec. The health technology assessment search included the assessment of the National Institute for Health and Care Excellence (NICE), the Scottish Medicines Consortium, the Pharmaceutical Benefits Advisory Committee, and the Canadian Agency for Drugs and Technologies in Health submissions for nusinersen. Key papers published after April 30, 2019 until the time of writing were included in the discussion to provide context to the results.

Selection of relevant studies

A search strategy (**Table S1**) was developed based on the population, interventions, comparison, outcomes, and study design (PICOS) framework. Searches were conducted for the broad SMA population and studies published in English including patients with type 1, 2, and 3 SMA were selected for inclusion in the SLR; this also includes a "mixed SMA type" population that featured types 1–3 SMA. Inclusion criteria included prospective cohort studies, retrospective cohort studies, RCTs, and single-arm trials. Cross-sectional studies, case series, case reports, and congress abstracts were excluded.

A risk of bias (ROB) assessment was carried out on studies included in the analysis to evaluate their methodologic quality and the strength of resulting evidence. For RCTs, the ROB tool used was taken from the NICE single technology appraisal template,[41] which has seven domains for assessment: random sequence generation, allocation concealment, comparability of groups, blinding, imbalance in dropouts, selective reporting, and incomplete reporting.[41] For observational studies, the Quality in Prognosis Studies (QUIPS) tool was used.[42] This was recommended in the latest systematic review guidance[43] and comprises six bias domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. The responses to each of the six domains were taken together to inform the judgment of ROB as high, moderate, or low.[42] Observational studies were considered the most reliable if they met the criteria of at least three of six QUIPS domains scoring at a low ROB.

Evidence synthesis

Prognostic factors are measures that are associated with changes in prognosis in the natural course of the disease.[44] Evidence of prognostic factors can be extracted from interventional and observational studies.

Treatment-effect modifiers are factors that are predictive of an improved response to a given therapy.[45] A treatment-effect modifier can be identified by comparing the effect of factors in treated vs. untreated populations. RCTs are most suitable to evaluate whether a factor is a treatment-effect modifier, as they provide the strictest approach to determine a cause and effect relationship (e.g., a forest plot and test for interaction).[46-48]

A distinction was made between studies on populations treated with disease-modifying therapies (DMTs), such as nusinersen, risdiplam and onasemnogene abeparvovec, and studies reporting on the natural course of the disease when patients receive supportive care only.

Effect sizes and uncertainty estimates (standard error, confidence intervals (CIs) and/or *P* values) were extracted for qualitative evidence synthesis. A broad threshold for statistical significance ($P < 0.2$) was adopted to evaluate association between outcomes and prognostic/predictive factors. Evidence was classified as showing a prognostic/predictive effect (i.e., $P < 0.2$) or that there is no evidence of a prognostic/predictive effect (i.e., $P \geq 0.2$). However, it should be noted that some studies only report whether associations are significant or not (typically using a $P < 0.05$ threshold). Synthesis included all the available evidence; however, studies with a low ROB were given more weight in the interpretation of the results than studies with higher ROB.

Similarly, less weight was given to the study which reported data on olesoxime.[49] This study failed to reach its primary end point, and no significant effects on motor and respiratory functions were observed in the open-label extension study (A Study to Evaluate Long Term Safety, Tolerability, and Effectiveness of Olesoxime in Patients With Spinal Muscular Atrophy (SMA) (OLEOS)),[50] resulting in the discontinuation of the study program. As olesoxime is no longer a possible treatment option for SMA, data from this study is reported for completeness but not discussed.

Results

Included studies

The preferred reporting items for systematic reviews and meta-analyses diagram is shown in **Figure S1**. A total of 1,483 database records were identified from the Embase and MEDLINE

database searches for the broad SMA population. After removing duplicates, 1,450 records were screened by two reviewers for inclusion in the literature review; 1,359 records were excluded. One additional record was added from the supplementary searches.[51] The remaining 92 records then underwent full-text screening to assess for eligibility.

Finally, 31 studies were identified for inclusion in this literature review (**Table 1**). Five studies reported on the effects of DMTs, and 26 studies reported on the natural course of the disease when patients received best supportive care (BSC) only. Three studies were RCTs, and 28 studies were observational studies, which included one indirect comparison analysis. Twelve studies reported data from type 1 SMA only, 7 studies reported data from individuals with types 2 and 3 SMA, and the remaining 12 reported data from patients in mixed SMA type populations that included all SMA types. Interventions received in these studies included nusinersen ($n = 4$), nusinersen vs. onasemnogene abeparvovec ($n = 1$), olesoxime ($n = 1$), tracheostomy ($n = 1$), noninvasive respiratory muscle aid ($n = 1$), valproic acid (VPA; $n = 1$), and unspecified ($n = 23$).

ROB in included studies

An overview of ROB scoring for included studies is shown in **Table 1**, and the full ROB assessment is provided in **Table S2**. The three RCTs identified through the literature search were determined to be at low ROB, with the nusinersen (A Study to Assess the Efficacy and Safety of Nusinersen (ISIS 396443) in Participants With Later-onset Spinal Muscular Atrophy (SMA) (CHERISH))[52] and olesoxime[49] studies in types 2 and 3 SMA being identified as low bias across all seven NICE domains, and the nusinersen study (A Study to Assess the Efficacy and Safety of Nusinersen (ISIS 396443) in Infants With Spinal Muscular Atrophy (ENDEAR)) in type 1 SMA[53] at low ROB in six of the seven domains.

Eleven observational studies were considered to be at a low ROB (**Table 1**). The remaining 17 observational studies were judged to be of a lower methodologic quality due to having more than three QUIPS domains at high or moderate ROB (**Table S2**). The quality of these studies should be considered when interpreting the findings. Evidence from low-ROB studies can be found in the main manuscript (**Tables 2–4**), and evidence from high-ROB studies can be found in the (**Tables S3–S6**) accompanying this manuscript.

Factors that affect survival. Factors and their association with survival outcomes are reported in **Table 2** (low ROB) and **Table S3** (high ROB).

Genotype. The literature on genotype factors focuses on two genes: *SMN2* and NLR Family Apoptosis Inhibitory Protein (*NAIP*).

The prognostic effect of *SMN2* copy number on survival was assessed in one low-ROB natural history study in a mixed SMA type population.[54] A positive association was reported between survival and higher *SMN2* copy numbers: Two *SMN2* copies were associated with a median survival of 6 months, whereas three or four *SMN2* copies were associated with longer survival (**Table 2**).[54] One high-ROB natural history study in a mixed population reported consistent findings and a hazard ratio (HR) of 0.179 ($P < 0.05$) for increased numbers of *SMN2* copies when comparing survival data from patients with one, two, three, and four/five copies of *SMN2* (**Table S3**).[55]

Results from high-ROB studies that investigated the effect of *SMN2* copy number on survival in untreated infants with type 1 SMA were also consistent with the studies above, with higher copy number being associated with increased survival (**Table S3**).[6,18,56]

The SLR did not find any studies that assess whether *SMN2* copy number has any predictive effect on survival outcomes in treated patients: In fact, all studies that evaluated the effect of a DMT in type 1 SMA enrolled infants with two copies of *SMN2* only.[53]

The presence of the *NAIP* gene was also associated with increased median survival in infants with type 1 SMA in the same low-ROB study that evaluated the effect of *SMN2* copy number, with 6, 13, and 53 months median survival observed in infants with no copies, one copy, and two copies of *NAIP*, respectively (**Table 2**).[54] However, statistical significance between subgroups was not tested. The authors also considered a mixed SMA type population and found lower *NAIP* copy numbers (0 vs. 2 copies and 1 vs. 2 copies) to be associated with greater mortality (0 vs. 2 copies: odds ratio for mortality 19.16; 1 vs. 2 copies: odds ratio for mortality 3.34, both at $P < 0.0001$).[54]

Similar to *SMN2* copy number, the SLR did not find any studies evaluating the predictive value of the *NAIP* gene on survival outcomes following treatment with DMTs.

Disease severity/symptoms. One low-ROB natural history study in type 1 SMA found an association between age of symptom onset and survival, reporting prolonged survival in infants with a later onset of disease: When prenatal symptom onset was compared with an onset of symptoms at 1, 2, 3, 4, and 5 months of age, it was observed that median survival time increased from 6 months when symptoms were present at birth, up to 40 months when symptom onset occurred at 5 months ($P = 0.002$; **Table 2**).[28] Results from three additional high-ROB studies[56-58] were consistent with these findings, with one study estimating the reduction in the risk of death for every additional month that infants are without symptoms to be as high as 40% (HR = 0.6; $P < 0.001$, **Table S3**).[58]

The prognostic effect of SMA type on survival outcomes was evaluated in one low-ROB natural history study in a mixed population, reporting reduced survival in type 1 SMA (**Table 2**).[28] Consistent observations were reported in six high-ROB studies (**Table S3**),[30,55,57,59-61] with the shortened median survival for patients with type 1 SMA varying between 5 months[61] and 13.6 months.[59] However, there was no evidence that SMA subtype is a prognostic factor in type 1 SMA: A high-ROB study, conducted in a small population, observed similar trends in median survival across subtypes 1b and 1c.[18]

One low-ROB study reported that symptoms such as poor motor function and respiratory function at baseline were associated with poorer survival outcomes in untreated infants with type 1 SMA. Infants with signs of the disease in the neonatal period (HR = 2.11 (95% CI: 0.95, 4.69); $P = 0.065$), respiratory distress at birth (HR = 4.10 (95% CI: 1.02, 16.40); $P = 0.046$), and reduced

Table 1 Included studies

	Author, year (reference)	Study design	Sample size	Follow-up duration	Treatment	Age, mean (range)	Country	NICE/ QUIPS ROB assessment
Studies in Type 1 SMA population	Aragon-Gawinska, 2018[67]	POS	33	6 m	Nusinersen	21.3 m (8.3–113.1)[a]	France	Low
	D'Amico, 2008[62]	ROS	38	NR	Unspecified	NR	Italy	Low
	Finkel, 2017 ENDEAR[53]	RCT (Phase 3, DB)	122	13 m	Nusinersen vs. sham procedure	Nusinersen: 163 d (52–242)[a] Placebo: 181 d (30–262)[a]	Multinational	Low
	Pechmann, 2018[68]	POS	61	6 m	Nusinersen	21.08 m (1–93)[a]	Germany	Low
	Dabbous, 2019[64]	ITC	92	NR	Onasemnogene abeparvovec vs. nusinersen	Onasemnogene abeparvovec: 3.4 m (0.9–7. 9)[a] Nusinersen: 5.3 m (1.7–7.9)[a]	Multinational	High
	De Sanctis, 2018[69]	ROS	20	NR	Unspecified	NR	Italy	High
	Feldkoetter, 2002[6]	ROS	113	NR	Unspecified	NR	Germany	High
	Finkel, 2014[18]	POS	79	36 m	Unspecified	Type 1b SMA (median): 2.5–184 m[b] Type 1c SMA (median): 6–78 m[b]	US	High
	Gregoretti, 2013[63]	ROS	194	NR	TV vs. continuous NRA	NR	Italy	High
	Kaneko, 2017[26]	ROS	47	NR	Unspecified	7 m to 57 y[b]	Japan	High
	Oskoui, 2007[58]	POS	143	49.9 m	Unspecified		US	High
	Rudnik-Schoneborn, 2009[56]	ROS	66	NR	Valproic acid	NR	Germany	High
Studies in Types 2 and 3 SMA population	Bertini, 2017[49]	RCT (Phase 2, DB)	165	24 m	Olesoxime vs. placebo	Olesoxime: 9.1 y (SD: 5.5) Placebo: 11.2 y (SD: 6.0)	Multinational	Low
	Mercuri, 2018 CHERISH[52]	RCT (Phase 3, DB)	126	15 m	Nusinersen vs. sham	Nusinersen: 4 y (range: 2–9) Sham: 3 y (range: 2–7)	Multinational	Low
	Mazzone, 2013[74]	POS	38	12 m	Unspecified	14.07 y (SD: 12.43)	Europe (6 countries)	Low
	Mercuri, 2016[73]	ROS	268	12 m	Unspecified	10.65 y (range: 2.5–55.5)	US, Italy, UK, Belgium	Low
	Montes, 2018[70]	POS	73	108 m	Unspecified	13.5 y (SD: 12.4)	US, Italy, UK	Low
	Pera, 2019[71]	POS	114	12 m	Unspecified	13.3 y (SD: 10.1)	US, Italy, UK	Low
	Kaufmann, 2012[66]	POS	79	25 m	Unspecified	11.3 y (SD: 9.4)	US	High

(Continued)

Table 1 (Continued)

	Author, year (reference)	Study design	Sample size	Follow-up duration	Treatment	Age, mean (range)	Country	NICE/ QUIPS ROB assessment
Studies in a mixed SMA population (Types 1, 2 and 3 SMA)	Farrar, 2013[28]	ROS	70	NR	Unspecified	NR	Australia	Low
	Swoboda, 2005[65]	POS	89	NR	Unspecified	Type 1 SMA: 18.9 m (1.08–263)[b] Type 2 SMA: 87.1 m (0.43–589)[b] Type 3 SMA: 146.6 m (28.4–604.8)[b]	US	Low
	Qu, 2015[54]	POS	232	NR	Unspecified	NR	China	Low
	Vuillerot, 2013[72]	ROS	112	21.6 m	Unspecified	Type 2 SMA: 11.5 y (SD: 5) Type 3 SMA: 18.7 y (SD: 12.3)	France, Belgium, Switzerland	Low
	Belter, 2018[59]	ROS	1966	NR	Unspecified	NR	US	High
	Bladen, 2014[17]	ROS	5068	NR	Unspecified	NR	North America, Australasia, Europe	High
	Chung, 2004[30]	POS	83	NR	Unspecified	Type 2 SMA: 0.96 y (SD: 0.58) Type 3a SMA: 1.31 y (SD: 0.62) Type 3b SMA: 6.93 y (SD: 8.03)	Hong Kong	High
	Ge, 2012[57]	ROS	237	6 m	Unspecified	Type 1 SMA: 5.3 y (SD: 4.7)[b] Type 2 SMA: 12.8 y (SD: 10.0)[b] Type 3 SMA: 32.8 y (SD: 27.7)[b]	China	High
	Madrid Rodríguez, 2015[60]	ROS	37	NR	Unspecified	NR	Spain	High
	Mannaa, 2009[61]	ROS	40	NR	Unspecified	NR	US	High
	Petit, 2011[55]	ROS	103	NR	Unspecified	NR	France	High
	Yuan, 2015[98]	ROS	132	49 m	Unspecified	Type 1 SMA: 7.8 y (SD: 5.7)[b] Type 2 SMA: 36.2 y (SD: 23.0)[b] Type 3 SMA: 110.3 y (SD: 63.2)[b]	China	High

All studies included in the systemic review. Studies were divided into Type 1 and Types 2 and 3 SMA populations and mixed SMA populations that reported on patients with Types 1, 2 and 3 SMA.
d, days; DB, double-blind; ITC, indirect treatment comparison; m, months; NICE, National Institute of Health and Care Excellence; NR, not reported; NRA, non-invasive respiratory muscle aid; POS, prospective observational study; QUIPS, Quality in Prognosis Studies; RCT, randomized controlled trial; ROB, risk of bias; ROS, retrospective observational study; SMA, spinal muscular atrophy; SD, standard deviation; TV, tracheostomy and invasive mechanical ventilation; y, years.
[a]Age at treatment initiation. [b]Age at enrollment/first visit.

Table 2 Factors and their association with survival reported in low-ROB studies

Factor		Study	Treatment	Subgroup		*N*	Outcome	Median survival in months (95% CI) unless otherwise specified	HR (95% CI) unless otherwise specified	Statistical significance between subgroup (*P*-value)
Genotype	*SMN2* copies (M)	Qu, 2015[54]	Unspecified	2 copies		66	Survival	6 (mean: 12.4; 7.5–17.3)	OR: 186 (19.08, 1812.68)	<0.0001
				3 copies		153		NA (mean: 184; 172–196)	OR: 1.02 (0.12, 8.54)	>0.05
				4 copies		13		NA (mean: 226; 186–266)	Reference	
	NAIP copies (E)	Qu, 2015[54]	Unspecified	0 copies		28	Survival	6 (NR)	NR	NR
				1 copy		66		13 (NR)		
				2 copies		12		53 (NR)		
	NAIP copies (M)		Unspecified	0 copies		35	Survival	7 (mean: 34; 20–48)	OR: 19.16[a] (6.23, 58.93)	<0.0001
				1 copy		145		NA (mean: 132; 113–151)	OR: 3.34[a] (1.33, 8.93)	<0.0001
				2 copies		52		NA (mean: 219; 197–241)	Reference	
Disease severity/ symptoms	Age at symptom onset (E)	Farrar, 2013[28]	Unspecified	Prenatal		7	Survival	6 (NR)	NR	0.002
				1 month		4		6.5 (NR)		
				2 months		1		8 (NR)		
				3 months		1		39 (NR)		
				4 months		3		35 (NR)		
				5 months		3		40 (NR)		
	SMA type (M)	Farrar, 2013[28]	Unspecified	Type 1		20	Survival	7.4 (3; 56)	NR	NR
				Type 2		31		NA (NR)		
				Type 3		19		NA (NR)		
	Symptoms (E)	D'Amico, 2008[62]	None	Neonatal neurologic symptoms	Absence	17	Survival	NR	Reference	0.065
					Presence	21		NR	2.11 (0.95, 4.69)	
				Head/trunk control	Absence	27		NR	Reference	<0.0001
					Presence	11		NR	0.11 (0.03, 0.32)	
				Respiratory distress at birth	No	33		NR	Reference	0.046
					Yes	5		NR	4.1 (1.02, 16.4)	
				Reduced fetal movements	No	28		NR	Reference	0.895
					Yes	10		NR	1.07 (0.39, 2.97)	

(Continued)

Table 2 (Continued)

Factor		Study	Treatment	Subgroup		*N*	Outcome	Median survival in months (95% CI) unless otherwise specified	HR (95% CI) unless otherwise specified	Statistical significance between subgroup (*P*-value)
Care-related factors	Nusinersen (E)	Finkel, 2017 ENDEAR[53]	Nusinersen vs. sham procedure	Nusinersen		80	EFS[b]	61%	0.53 (0.32, 0.89)	0.005
				Sham control		41		32%	Reference	
				Nusinersen		80	EFS[c]	61%	0.53 (0.32, 0.89)	NR
				Sham control		41		32%	Reference	
				Nusinersen		NR	EFS[d]	NR	0.70 (0.30, 1.60)	NR
				Sham control		NR		NR	Reference	
				Nusinersen		80	EFS[e]	NR	0.53 (0.31, 0.90)	NR
				Sham control		41		NR	Reference	
				Nusinersen		80	OS	84%	0.37 (0.18, 0.77)	0.004
				Sham control		41		61%	Reference	
				Nusinersen		80	No permanent ventilation	78%	0.66 (0.32, 1.37)	0.13
				Sham control		41		68%	Reference	
	Disease duration (E)	Finkel, 2017 ENDEAR[53]	Nusinersen	Below median (≤13.1 w) at screening	Nusinersen	39	EFS[c]	NA	0.24 (0.1, 0.58)	<0.001
					Sham control	21		25.4 w	Reference	
				Above median (>13.1 w) at screening	Nusinersen	41	EFS[c]	27.4 w	0.84 (0.43, 1.67)	0.4
					Sham control	20		19 w	Reference	
Demographics	Gender (E)	D'Amico, 2008[62]	Unspecified	Male		23	Survival	NR	Reference	0.777
				Female		15		NR	1.12 (0.52, 2.37)	

CI, confidence interval; E, early-onset (Type 1) population; EFS, event-free survival; HR, hazard ratio; M, mixed SMA type population; NA, not available; NAIP, NLR Family Apoptosis Inhibitory Protein; NR, not reported; OR, odds ratio; OS, overall survival; ROB, risk of bias; SMA, spinal muscular atrophy; *SMN2*, survival of motor neuron 2; w, weeks.

[a]OR for mortality. [b]Defined as time to death or the use of permanent assisted ventilation (tracheostomy or ventilatory support for ≥16 hours per day for >21 continuous days in the absence of an acute reversible event). [c]From a Cox regression adjusted for each infant's disease duration at screening. [d]EFS was re-evaluated in the subset of infants who received at least the first four doses of nusinersen or sham procedure and had baseline and at least Day 183 efficacy assessments with no significant protocol deviations. [e]In this analysis, infants who had been previously classified as on permanent ventilation, but who were found at the end of study to be using ventilation for <16 hours per day, were reclassified as not being on permanent ventilation.

Table 3 Genotype factors and their association with motor function in low-ROB studies

Factor	Study/ROB	Treatment	Subgroup		*N*	Outcome	Median change in score (range) unless otherwise specified		Statistical significance between subgroups (*P*-value)
SMN2 copy number (E)	Aragon-Gawinska, 2018[67]	Nusinersen	2 copies		14	HINE-2 (6 months f/u)	1.5 (–1, 4)		>0.05
			3 copies		16		1.5 (0, 9)		
			2 copies		12	CHOP-INTEND (6 months f/u)	3.5 (–2, 11)		>0.05
			3 copies		10		4 (–2, 14)		
	Pechmann, 2018[68]	Nusinersen	≤2 copies		38	CHOP-INTEND (6 months f/u)	Mean: 8.1 (SD: 7.0)		>0.01
			≥3 copies		20		Mean: 8.2 (SD: 5.3)		
SMN2 copy number (M)	Swoboda, 2005[65]	Unspecified	1 copy		1	Functional status: Unable to sit	0%		<0.001
			2 copies		22		55%		
			3 copies		41		17%		
			4 copies		14		14%		
			5 copies		3		0%		
			1 copy		1	Functional status: Sits unsupported	0%		
			2 copies		22		4%		
			3 copies		41		68%		
			4 copies		14		79%		
			5 copies		3		33%		
			1 copy		1	Functional status: Walks/ cruises	0%		
			2 copies		22		0%		
			3 copies		41		15%		
			4 copies		14		7%		
			5 copies		3		67%		
SMN2 copy number (L)	Mercuri, 2018 CHERISH[52]	Nusinersen	2 copies	Nusinersen	9	Change from baseline in HFMSE score to Month 15	3.3	5.6[a]	NR
				Sham			–2.3		
			3 copies	Nusinersen	87		4.1	4.4[a]	
				Sham			–0.3		
			4 copies	Nusinersen	2		5.0	15[a]	
				Sham			–10.0		
			Unknown	Nusinersen	2		2.0	NC	
				Sham			NC		

CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; E, early-onset (Type 1 population); f/u, follow-up; HFMSE, Hammersmith Functional Motor Scale – Expanded; HINE-2, Hammersmith Infant Neurological Examination, Section 2; L, later-onset (Types 2/3) population; M, mixed SMA type population; NC, not calculated; NR, not reported; ROB, risk of bias; SD, standard deviation; *SMN2*, survival of motor neuron 2.

[a]Treatment difference.

Table 4 Disease severity/symptom factors and their association with motor function in low-ROB studies

Factor	Study	Treatment	Subgroup	*N*	Outcome	Mean change (95% CI) unless otherwise specified	Statistical significance between subgroups (*P*-value)
Disease severity (L)	Bertini, 2017[49]	Olesoxime vs. placebo	< median MFM D1+D2 score at baseline	79	MFM D1+D2 24 m change Olesoxime vs. placebo diff	2.97 (−0.36, 6.31)[a]	NR
			≥ median MFM D1+D2 score at baseline	81		1.25 (−1.64, 4.15)[a]	
SMA type (L)	Bertini, 2017[49]	Olesoxime vs. placebo	Type 2	113	MFM D1+D2 24 m change Olesoxime vs. placebo diff	2.06 (−0.78, 4.90)[a]	NR
			Type 3	47		2.06 (−0.83, 4.94)[a]	
			Type 2	113	HFMS 24 m change Olesoxime vs. placebo diff	0.89 (−0.51, 2.29)[a]	NR
			Type 3	47		0.72 (−1.72, 3.16)[a]	
SMA subtype (L)	Montes, 2018[70]	Unspecified	Type 3a	52	6MWD mean 12 m change	−8.5 meters (−15.2, −1.7)	0.78
			Type 3b	21		−6.6 meters (−17.7, 4.4)	
	Pera, 2019[71]	Unspecified	Type 2	60	RULM 12 m change	−0.45 (2.9)	0.91
			Type 3 (non-ambulant)	22		−0.23 (2.7)	
			Type 3 (ambulant)	32		−0.34 (3.0)	
Ambulatory status (L)	Mazzone, 2013[74]	Unspecified	Pearson *r* correlation	38	Change in 6MWD over 12 m	0.19	0.30
Ambulatory status (L)	Mercuri, 2016[73]	Unspecified	Yes	268	HFMSE	0.83 (NR)	0.029
			No	10		−0.84 (NR)	

6MWD, 6-minute walk distance; CI, confidence interval; D, domain; diff, difference; HFMS, Hammersmith Functional Motor Scale; diff, difference; HFMSE, Hammersmith Functional Motor Scale – Expanded; L, later-onset SMA (Types 2/3) population; m, months; MFM, Motor Function Measure; NR, not reported; ROB, risk of bias; RULM, Revised Upper Limb Module; SMA, spinal muscular atrophy; y, years.
[a]Results are from a mixed-effects repeated measure model.

fetal movements (HR = 1.07 (95% CI: 0.39, 2.97); P = 0.895) were found to have reduced survival, even if associations were not always significant. Likewise, the achievement of early motor milestones was found to be a positive prognostic factor for survival in type 1 SMA, with head/trunk control being associated with longer survival compared with infants who did not achieve this milestone (HR = 0.11 (95% CI: 0.03, 0.32); P < 0.0001; **Table 2**).[62] Similarly, the involvement of brain stem motor neurons (determined by the presence of facial weakness or feeding/swallowing difficulties or tongue/speech symptoms) was found to be associated with poorer survival outcomes in a single high-ROB study (**Table S3**).[55]

Care-related factors. In the ENDEAR RCT, infants who received nusinersen were significantly more likely to be alive without the need for permanent ventilation at the end of the study than those treated with placebo (**Table 2**; HR = 0.53, 95% CI: 0.32, 0.89; P = 0.005), providing evidence that treatment with a DMT such as nusinersen is associated with improved outcomes in type 1 SMA.[53]

The study also compared the effect of treatment in infants with a disease duration above and below the median of 13.1 weeks at time of initiation of treatment. Event-free survival was significantly improved (HR = 0.24 (95% CI: 0.1, 0.58); P < 0.001) for patients who received nusinersen compared with sham control in patients with a shorter disease duration.[53] Conversely, there was no evidence (HR = 0.84 (95% CI: 0.43, 1.67); P = 0.4) of a treatment effect in infants having a disease duration above 13.1 weeks at screening. The P value for the interaction term (test for effect modification) was not reported,[53] but the data seem to suggest that disease duration has a significant modifying effect on the efficacy of nusinersen.

Since the publication of SoC guidelines in 2007,[31] there have been significant advances in BSC for SMA, with an increased drive in the use of respiratory and nutritional support. Several high-ROB observational studies documented the positive effect of clinical supportive care on survival (**Table S3**).[56,58,63]

Two high-ROB studies found an association between treatment with VPA or onasemnogene abeparvovec and survival (**Table S3**).[56,64]

Demographic factors. One low-ROB natural history study did not find any difference in survival between female and male patients with type 1 SMA (**Table 2**),[62] whereas some high-ROB studies found some associations between gender and survival outcome (**Table S3**).[18,56,57,62]

Factors that affect motor function. Factors and their associations with motor function are reported in **Tables 3–6** (low-ROB studies) and **Tables S4–S7** (high-ROB).

Genotype. This SLR did not find any studies reporting on the effect of *SMN2* copy number on motor function in the natural history of type 1 SMA.

In a mixed SMA type natural history population (types 1, 2, and 3 SMA), *SMN2* copy number was found to be a prognostic factor associated with improved functional status (P < 0.001), with a greater proportion of infants with higher *SMN2* copy numbers able to sit unassisted or cruise/walk (**Table 3**).[65] *SMN2* copy number and its effects on motor functions in types 2 and 3 SMA were reported also in one high-ROB study. Functional motor declines over time were visually steeper in individuals with three copies of *SMN2* compared with individuals with four to five copies of *SMN2*, but these differences were not significant (**Table S4**).[66]

In the ENDEAR RCT, evaluating treatment with nusinersen in type 1 SMA, all infants had two copies of *SMN2*;[53] it was therefore not possible to assess whether *SMN2* copy number had any effect upon treatment outcomes related to motor function.

Two low-ROB studies investigated the effect of *SMN2* copy number on treatment outcomes in a type 1 SMA population treated with nusinersen (**Table 3**).[67,68] Neither study reported statistical associations between *SMN2* copy number and motor function improvements. When comparing infants aged >7 months with two vs. three copies, no difference was observed in either the median change of the Hammersmith Infant Neurological Examination, Section 2 total score (1.5 on average for both groups) or the median change in score on the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) over 6 months (two copies: 3.5 points; three copies: 4.0 points).[67] In a similar study that included children as young as 1 month, greater absolute effects were observed over the same time span,[68] but identical improvements in the CHOP-INTEND scores were observed in children with two or fewer *SMN2* copies (8.1-point improvement) and children with three or more copies (8.2-point improvement) on average.

In the CHERISH RCT,[52] the change in Hammersmith Functional Motor Scale –Expanded (HFMSE) scores from baseline to 15 months was evaluated in nusinersen and sham groups stratified by *SMN2* copy number. The median age of patients at the time of study was 4 years (range: 2-9 years). Individuals in the nusinersen arm with three copies of *SMN2* had a larger mean change in HFMSE score when compared with individuals with three *SMN2* copies who received sham (**Table 3**; treatment difference: 4.4 points). Most of the CHERISH participants had three copies of *SMN2*, which makes inferences about the efficacy of nusinersen across different SMN2 copy number populations difficult.

Disease severity/symptoms. The SLR did not find any low-ROB studies evaluating the prognostic effect of disease severity on motor function outcomes in type 1 SMA, but one high-ROB natural history study investigated the effect of the severity of the SMA phenotype (classified as mild, typical, or severe) and reported greater monthly decline in CHOP-INTEND score in patients with more severe SMA (**Table S5**).[69] When severity was defined by subtype (type 1b vs. 1c), greater declines in CHOP-INTEND score were reported in infants with type 1b SMA in a high-ROB study, although this difference was not significant (**Table S5**).[18]

The ENDEAR study did not report on efficacy of nusinersen by disease severity.

Two low-ROB natural history studies examined the effect of subtype on motor function outcomes in types 2 and 3 SMA and did not find any differences in motor decline across subtypes at month 12, although declines were more pronounced in less severe

Table 5 Care-related factors and their association with motor function in SMA in low-ROB studies

Factor	Study	Treatment	Subgroup	*N*	Outcome	Mean change (95% CI) unless otherwise specified	Effect size, between subgroups (95% CI)	Statistical significance between subgroups (*P*-value)
Treatment (E)	Finkel, 2017 ENDEAR[53]	Nusinersen vs. sham control	Nusinersen	51	Motor milestone response[a]	41%	NR	<0.001
			Sham control	27		0%		
			Nusinersen	51	Motor milestone response[b]	43%	NR	<0.001
			Sham control	27		0%		
			Nusinersen	51	Motor milestone response[c]	37	NR	<0.001
			Sham control	27		0%		
			Nusinersen	52	Motor milestone response[d]	40%	NR	<0.001
			Sham control	30		0%		
			Nusinersen	52	Motor milestone response[e]	37%	NR	<0.002
			Sham control	30		0%		
			Nusinersen	73	Motor milestone response[a]	51%	NR	NR
			Sham control	37		0%		
Treatment (L)	Bertini, 2017[49]	Olesoxime	Olesoxime	103	MFM D1+D2 change at Month 24	0.18 (–1.30, 1.66)	NR	0.068
			Placebo	57		–1.82 (–3.68, 0.04)		
	Mercuri, 2018 CHERISH[52]	Nusinersen vs. sham control	Nusinersen	84	HFMSE change at Month 15	3.9 (3.0, 4.9[f]	NR	NR
			Sham control	42		–1.0 (–2.5, 0.5)[f]		
			Nusinersen	84	HFMSE responder at Month 15[g]	57%	NR	<0.001
			Sham control	42		26%		
Age at treatment initiation (E)	Pechmann, 2018[68]	Nusinersen	≤7 months	17	CHOP-INTEND	+14.4 (SD: 9.2)	NR	NR
			>7 months	44		+7.0 (SD: 6.6)	NR	NR
			Effect of 1-month delay	61	CHOP-INTEND	NR	–0.146 (–0.227, –0.006)	0.0006

(Continued)

Table 5 (Continued)

Factor	Study	Treatment	Subgroup		N	Outcome	Mean change (95% CI) unless otherwise specified	Effect size, between subgroups (95% CI)	Statistical significance between subgroups (*P*-value)
Age at treatment initiation (L)	Bertini, 2017[49]	Olesoxime	Olesoxime vs. placebo		160	MFM D1+D2 24 m change continuous covariate	NR	NR	0.25
			3–<6 y	Olesoxime	35	MFM D1+D2 24 m change	+0.76[h]	NR	0.75
				Placebo	13		+0.01[h]		
			6–15 y	Olesoxime	54		+0.70[h]	NR	0.036
				Placebo	25		−2.91[h]		
			>15 y	Olesoxime	14		−0.65[h]	NR	0.96
				Placebo	19		−0.63[h]		
			<6 y		48	MFM D1+D2 24 m change Olesoxime vs. placebo diff	0.75 (−3.86, 5.35)		
			≥6 y		112		2.21 (0.21, 4.62)		
			<6 y		48	HFMS 24 m change Olesoxime vs. placebo diff	1.54 (−1.25, 4.33)		
			≥6 y		112		0.68 (−0.71, 2.06)		

CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CI, confidence interval; D, domain; diff, difference; E, early-onset (Type 1) SMA population; diff, difference; HFMS, Hammersmith Functional Motor Scale; HFMSE, Hammersmith Functional Motor Scale – Expanded; HINE-2, Hammersmith Infant Neurological Examination, Section 2; L, later-onset (Types 2/3) SMA population; m, months; MFM, Motor Function Measure; NR, not reported; ROB, risk of bias; SD, standard deviation; SMA, spinal muscular atrophy; y, years.
[a]Infants were considered to have a motor milestone response if they met the following two criteria: improvement in ≥1 category of the HINE-2 (i.e., an increase in the score for head control, rolling, sitting, crawling, standing, or walking of ≥1 point; an increase in the score for kicking of ≥2 points; or achievement of the maximal score for kicking) and more categories with improvement than categories with worsening. [b]Defined as the same as [a]regarding the degree of improvement and worsening, but the second part of the definition required infants to acquire ≥1 milestone overall compared with baseline instead of requiring more categories with improvement than worsening. [c]Defined as a 2-point increase in motor milestone score from baseline. [d]Same as [a], but includes all patients (i.e., including deaths and withdrawals). [e]Same as [b], but includes all patients (i.e., including deaths and withdrawals). [f]Least squares mean change from baseline in HFMSE score after 15 months of treatment. [g]A HFMSE response is defined as an increase from baseline in the HFMSE score of ≥3 points. [h]Extracted from figure 3 in Bertini et al.[49]

Table 6 Demographic factors and their association with motor function in low-ROB studies

Factor	Study	Treatment	Subgroup	*N*	Outcome	Mean change (95% CI) unless otherwise specified	Statistical significance between subgroups (*P*-value)
Age (L)	Mazzone, 2013[74]	Unspecified	NR	38	6MWD at 12 m	Pearson *r* correlation *r* = 0.04	0.80
	Mercuri, 2016[73]	Unspecified	<5 y	200	HFMSE 12 m change (Non-ambulant Type 2)	0.04 (0.34)	0.048
			5–14 y			−0.96 (0.24)	
			≥15 y			−0.35 (0.43)	
			<5 y	68	HFMSE 12 m change (Ambulant Type 3)	0.56	0.34
			5–14 y			−0.61	
			≥15 y			−1.2	
			Age class (assumed <5 vs. 5–14 vs. ≥15 y)	NR	HFMSE 12 m change (Type 3a)	NR	0.067
			Age class (assumed <5 vs. 5–14 vs. ≥15 y)	NR	HFMSE 12 m change (Type 3b)	NR	0.80
	Montes, 2018[70]	Unspecified	<6 vs. 6–10 y	24; 24	6MWD Difference in rate of change at 12 m	−17.7 (−34.1, −1.4)	0.03
			<6 vs. 11–19 y	24; 10		−30.7 (−48.6, −12.7)	0.0009
			<6 vs. ≥20 y	24; 15		−19.6 (−38, −1.1)	0.04
			6–10 vs. 11–19 y	24; 10		−12.9 (−22.4, −3.4)	0.008
			6–10 vs. ≥20 y	24; 15		−1.8 (−13.8, 10.2)	0.77
			11–19 vs. ≥ 20 y	10; 15		11.1 (−2.5, 24.7)	0.11
			<6 y	24	6MWD Rate of change at 12 m	9.8 (−6.2, 25.9)	0.23
			6–10 y	24		−7.9 (−15.72, −0.1)	0.05
			11–19 y	10		−20.8 (−31.1, −10.6)	<0.0001
			≥20 y	15		−9.7 (−19.3, −0.1)	0.05
	Pera, 2019[71]	Unspecified	<5 y	114	RULM 12 m change (Type 2/3)	1.2 (4.7)	NR
			5–9 y			−0.3 (2.4)	
			10–14 y			−1.1 (2.6)	
			≥15 y			−0.6 (2.3)	
			<5 y	60	RULM 12 m change (Type 2)	0.9 (4.2)	0.21
			5–9 y			−0.9 (2.9)	
			10–14 y			−1.5 (2.9)	
			≥15 y			0.2 (1.8)	
			<5 y	22	RULM 12 m change (Non-ambulant Type 3)	NA	0.22
			5–9 y			1.0 (2.4)	
			10–14 y			−0.2 (2.9)	
			≥15 y			−1.7 (2.4)	
			<5 y	32	RULM 12 m change (Ambulant Type 3)	1.8 (5.8)	0.79
			5–9 y			−0.2 (1.0)	
			10–14 y			−1.4 (2.5)	
			≥15 y			−1.4 (2.7)	
Gender (L)	Bertini, 2017[49]	Olesoxime	Male	80	HFMS 24 m change Olesoxime vs. placebo diff	1.5 (−0.32, 3.33)	NR
			Female			0.72 (−1.02, 2.47)	
			Male	80	MFM D1+D2 24 m change Olesoxime vs. placebo diff	0.6 (−2.51, 3.70)	NR
			Female			3.05 (−0.11, 6.21)	
	Montes, 2018[70]	Unspecified	Female	73	6MWD	−5.9 (NR)	0.51
			Male			−10 (NR)	

(Continued)

Table 6 (Continued)

Factor	Study	Treatment	Subgroup	*N*	Outcome	Mean change (95% CI) unless otherwise specified	Statistical significance between subgroups (*P*-value)
Geographic location (L)	Mercuri, 2018 CHERISH[52]	Nusinersen	North America	64	LSM change from baseline in HFMSE score at Month 15 between nusinersen vs. sham	5.9 (3.4, 8.3)	NR
			Europe	32		5.6 (3.1, 8.0)	
			Asia-Pacific	4		NR	
			North America	64	≥3-point change in HFMSE (%) at Month 15 between nusinersen vs. sham	34.33 (8.45, 57.71)	NR
			Europe	32		29.87 (−8.18, 60.90)	
			Asia-Pacific	4		50.00 (−61.18, 98.74)	

6MWD, 6-minute walk distance; CI, confidence interval; D, domain; diff, difference; HFMS, Hammersmith Functional Motor Scale; HFMSE, Hammersmith Functional Motor Scale – Expanded; L, later-onset (Types 2/3) SMA population; LSM, least-squares mean; m, months; MFM, Motor Function Measure; NR, not reported; ROB, risk of bias; RULM, Revised Upper Limb Module; SD, standard deviation y, years.

types of the disease (**Table 4**).[70,71] These findings are consistent with observations in high-ROB natural history studies,[66,72] although one study found significantly better motor function outcomes in patients with type 3a SMA compared with patients with type 3b with regard to stair climbing (**Table S5**).[26]

An RCT of olesoxime in types 2 and 3 SMA found no effect of disease severity (defined by Motor Function Measure Domain 1 + Domain 2 total score) on outcomes (**Table 4**).[49]

The effect of ambulatory status at baseline on motor function (6-minute walk distance (6MWD), HFMSE, and GMFM (Gross Motor Function Measure) were assessed in two low-ROB natural studies in types 2 and 3 SMA (**Table 6**).[73,74] The first study reported that 6MWD at baseline had no significant correlation with change in 6MWD at month 12 (Pearson $r = 0.19$; $P = 0.30$) in a type 3 SMA population.[74] However, in the second, larger study, ambulation was found to be associated with better HFMSE outcomes after adjusting for baseline score and age class ($P = 0.029$): HFMSE score increased on average in ambulant individuals (+0.83) compared with a decrease in nonambulant individuals (−0.84).[73] Similarly, in a high-ROB study, ambulant individuals had a lower decline in mean motor function scores (GMFM and HFMSE) scores over a 3-year period compared with nonambulant individuals, although this difference was not found to be significant.[66]

Care-related factors. In type 1 SMA, no low-ROB studies report on the prognostic value of diagnosis time frame on natural history of disease. One high-ROB natural history study found no evidence to suggest that diagnosis time frame (>3 months before study enrollment vs. recent diagnosis) had a prognostic effect on 12-month changes in CHOP-INTEND (**Table S5**).[18] The SLR found no studies reporting on predictive value of diagnosis time frame on treatment with DMTs.

In types 2 and 3 SMA, there were no low-ROB studies evaluating the effect of non-SMN-targeting medications on motor function, with the exception of one RCT evaluating the efficacy of olesoxime (**Table 5**).[49] In a high-ROB study, treatment with medicines such as albuterol, carnitine, creatine, hydroxyurea, oral steroids, and VPA were associated with a greater decline in motor function score over a 3-year period (**Table S6**).[66]

Treatment with a DMT was predictive of improved motor milestone response in type 1 SMA: 41% (21/51) of infants randomized to nusinersen in ENDEAR compared with 0% (0/27) randomized to sham control achieved a motor milestone response ($P < 0.001$; interim analysis). In the final analysis, the percentage of children who achieved an increase of ≥4 points in CHOP INTEND score was greater in the nusinersen group than in the sham control (71% vs. 3%, $P < 0.001$).[53]

The ENDEAR study did not report on the effect of treatment initiation on motor functional end points, but age at treatment initiation was suggested to be a major determinant of the change from baseline in CHOP-INTEND scores in children treated with nusinersen in a low-ROB observational study,[68] where every month of delay in initiation of therapy negatively impacted results achieved at 6 months of follow-up (effect size for the change in CHOP-INTEND score: −0.146 (95% CI: −0.227, −0.006; $P = 0.0006$); **Table 5**).[68]

A high-ROB study found an association between treatment with onasemnogene abeparvovec and motor function (**Table S6**).[66]

Individuals with types 2 and 3 SMA in the CHERISH study (median age at treatment initiation: 4.0 years; range: 2–9 years) treated with nusinersen improved on their baseline HFMSE score by 3.9 points on average, while individuals randomized to the sham group lost on average 1.0 point relative to baseline after 15 months of treatment (**Table 5**).[52] At the end of the study, a greater proportion of individuals in the nusinersen group than in the placebo group demonstrated an increase of at least 3 points in their HFMSE score (57% vs. 26%; $P < 0.001$; **Table 5**).

Results in the CHERISH study were not stratified by age and there were no further studies in this SLR that evaluated the effect of nusinersen on motor function by age at treatment initiation in DMT-treated individuals with types 2 and 3 SMA. Age at treatment initiation was investigated in the phase II RCT investigating olesoxime and suggested that greater effects were seen in patients aged 6–15 years; however, the study did not meet the primary end point.[49]

Demographic factors. The prognostic effect of age at enrollment on the change in HFMSE, GMFM, Revised Upper Limb

Module (RULM), and 6MWD were investigated in four low-ROB observational studies (**Table 6**)[70,71,73,74] and one high-ROB study[66] in types 2 and 3 SMA (**Table S7**). All studies reported that younger individuals experienced improved motor function outcomes.

In terms of 6MWD, no significant linear association was observed between age and 1-year changes in ambulant type 3 SMA patients ($r = 0.04$, $P = 0.80$; **Table 6**).[74] However, children <6 years old tended to have slightly better scores compared with prepubertal patients, who had a more variable range and relatively higher risk of deterioration. In a second study,[70] annual gains in 6MWD were observed in the youngest age group (<6 years, 9.8-m increase in 1 year; $P = 0.23$), compared with adolescents (11–19 years, 20.8-m decrease; $P < 0.0001$) and adults (≥20 years, 9.7-m decrease; $P = 0.05$).

A significant difference was observed in the 12-month change in HMFSE score between different age groups of nonambulant individuals with type 2 SMA,[73] with scores increasing by 0.04 in younger patients (<5 years), and decreasing in intermediate (5 to 14 years) and older individuals (≥15 years) ($P = 0.048$). Similar results were observed in HFMSE scores in ambulant individuals with type 3 SMA,[73] though these results were not significant ($P = 0.34$). When ambulant children were separated into subtypes (3a or 3b), the change in HFMSE score was associated with age in type 3a children ($P = 0.067$) only.[73]

Among the three functional groups (type 2, type 3 ambulant, and type 3 nonambulant SMA), on average, RULM scores improved over the course of a year in younger patients and declined in patients >10 years.[71]

Results in the high-ROB study were consistent with the low-ROB data, with motor function declining less over time in younger individuals (**Table S7**).[66]

No studies report on the prognostic or predictive value of gender on motor function in type 1 SMA. Overall, there is no significant impact of gender on the natural history of types 2 and 3 SMA in both low-ROB (**Table 6**)[49,70] and high-ROB (**Table S7**)[24] studies. One low-ROB study found no evidence of predictive value of gender on treatment with olesoxime (**Table 5**).[49]

There were no low-ROB studies that compare natural history across geographies. One high-ROB study observed a significant effect on geographic location and the loss of ambulation, with mean age at loss of ambulation ranging from 9 years (Serbia) to 19 years (United Kingdom) (**Table S7**).[17] Patients in Germany/Austria, Switzerland, and the United Kingdom experienced longer median times of ambulation after diagnosis ($P = 0.014$) compared with patients in Argentina, Hungary, Ukraine, and Serbia.[17]

Geographic location was evaluated in one RCT (**Table 6**).[52] Due to small sample sizes in Asia, only data from Europe and North America could be compared. Results on the efficacy of nusinersen were similar across the two locations.[52]

Factors that affect respiratory function. Only two high-ROB studies investigated respiratory function in type 1[26] SMA and types 2 and 3 SMA (**Table S8**).[66]

In type 1 SMA, consistent with what was observed above, infants with head control had a longer median time to the introduction of respiratory support with tracheostomy positive pressure ventilation compared with patients without head control.

In types 2 and 3 SMA, there was no association between SMA type (type 2 vs. 3 SMA; $P = 0.61$; **Table S8**), ambulatory status ($P = 0.55$) or HFMSE score at baseline ($P = 0.36$) and change in forced vital capacity (FVC) after 3 years.[66] Higher *SMN2* copy numbers (4–5 copies vs. 2–3; **Table S8**; $P = 0.19$) were associated with a smaller decline in mean change in FVC, and a better baseline respiratory function (FVC ≥ 70%) was associated with a larger decline in mean change in FVC (**Table S8;** $P = 0.0007$). A prognostic effect on the change in FVC was found for patients taking SMA medication (albuterol, carnitine, creatine, hydroxyurea, oral steroids, and VPA) vs. those not taking medication, with treatment being associated with a larger decline in FVC after 3 years.

Discussion

This SLR aimed to identify prognostic factors and treatment-effect modifiers in types 1, 2, and 3 SMA.

Factors affecting survival

Studies evaluating the prognostic value of genetic factors in SMA natural history indicated that higher *SMN2* and *NAIP* copy number were associated with prolonged survival, although *SMN2* seems to be a stronger predictor of survival than *NAIP*.[54] Recent data have indicated that *NAIP* copy number does not predict clinical phenotype in SMA, thereby limiting its usefulness as a prognostic factor.[75] Based on the results of this review, *SMN2* copy number emerges indeed as a strong genetic prognostic factor for survival in natural history studies.

However, the genotype-phenotype relationship of *SMN2* copy number is not absolute;[8] a limited number of individuals who carry the mutation c.859G>C variant within *SMN2* do not appear to present the most severe form of SMA, regardless of *SMN2* copy number.[76]

Disease severity is also a key prognostic factor of survival in SMA. Clinically, disease severity is categorized by SMA type, which is based on the age of symptom onset. In this manuscript, an association between SMA type and survival was observed, with reduced survival in type 1 SMA.[28] Likewise, the early onset of symptoms[28] and also the presence of severe symptoms (such as respiratory distress at birth, reduced fetal movements, and the absence of head/trunk control)[62] were also associated with reduced survival. In total, regardless of how it is defined, it appears that clinical disease severity is a strong prognostic factor of survival. This finding is consistent with recently published data demonstrating that longer end point–free survival (defined as the need for mechanical ventilation) is associated with less severe SMA.[77]

High-ROB observational studies in individuals with type 1 SMA who have not received treatment with DMTs report a beneficial effect of supportive care interventions such as assisted ventilation[58,63] and nutritional support.[58] Indeed, SoC recommendations in 2007[31]have led to a measurable improvement in the survival of patients treated in subsequent years.[59] The role of supportive care alone may be under-represented in recent

literature as infants in clinical trials all received BSC; however, BSC does not result in acquisition of motor milestones in this condition.

While the importance of the implementation of SoC guidelines should not be diminished, the recent approval of DMTs has dramatically altered the prognosis of SMA. Nusinersen was investigated in two of the three RCTs included in this review. The ENDEAR study, the only RCT published in a type 1 SMA population, found that nusinersen treatment was associated with significantly prolonged survival.[53] Similar to findings from observational studies discussing symptom onset,[28] a shorter disease duration before nusinersen initiation was associated with prolonged survival in type 1 SMA.[53]

Improvements in survival in type 1 SMA have also been reported in response to treatment with other DMTs, not included in this SLR. Onasemnogene abeparvovec has also been associated with a significant increase in survival in an open-label study.[78] This study was identified in the literature search but was excluded during screening as it did not report CIs, statistical significance, or relative measures of treatment effect for subgroups. Significantly prolonged survival was observed in an open-label study in infants with type 1 SMA treated with risdiplam.[79]

Factors affecting motor function

Increasing *SMN2* copy number is prognostic of improved motor function in untreated individuals with type 1 SMA, with greater proportions of individuals achieving major motor milestones such as sitting and walking as *SMN2* copy number increases.[65]

However, in individuals treated with DMTs, *SMN2* genotype does not seem to have any predictive value in response to nusinersen treatment. In fact, two observational studies evaluating change in motor function observed near-identical trajectories in infants with type 1 SMA with two or fewer *SMN2* copies as in children with three or more copies of *SMN2*.[67,68] As many clinical trials only include individuals with a specified number of *SMN2* copies, it may be difficult to ascertain the true effect of *SMN2* copy number on motor function in an SMA patient population treated with DMTs until more real-world studies have been conducted.

Indeed, from the results in this SLR, we are unable to assess the predictive value of *SMN2* copy number on motor function outcomes with relation to DMT treatment, as in the ENDEAR study, all infants enrolled had two copies of *SMN2*.[53] Similarly, most (87%) individuals in the CHERISH study with types 2 and 3 SMA had three copies of *SMN2*,[52] which made evaluating the efficacy across *SMN2* copy number populations difficult. Consequently the CHERISH data indicated only a trend towards higher *SMN2* copy numbers being beneficial; they did not test for significance.[52] However, although this may be the case in patients with measurable clinical disease, in individuals treated at the presymptomatic stage, lower *SMN2* copies are associated with motor delay and bulbar symptoms.[80]

The effect of disease severity on motor function was only reported in high-ROB observational studies in type 1 SMA.[18,69] These studies indicated that more severe disease is associated with worse motor outcomes. Indeed, greater baseline motor function (and a clinical response to treatment) predicts an increased probability of acquiring a sitting position after 6 months of nusinersen treatment.[81]

In types 2 and 3 SMA the effect of disease severity is less obvious than in type 1 SMA. Although all low-ROB studies in our review report that increased disease severity was associated with poorer motor outcomes,[49,70,71,73,74] only one study examining ambulatory status (walkers vs. non walkers) in a mixed population reported significant findings ($P < 0.05$), with ambulant individuals having a better prognosis over 12 months.[73] This is to be expected as the types 2 and 3 SMA encompass a broad spectrum of functional abilities: Some individuals can sit independently but not stand, some can stand and others can walk, and some can lose these abilities over time.[34] Additionally, these studies report results over relatively short follow-up time frames (12–24 months). As there is large heterogeneity between individuals, these time scales may not be sufficient to capture differences in individuals who are declining more slowly.

Recent data in nusinersen-treated individuals reported that improvements in motor function are greater in individuals with type 3 SMA than in type 2 SMA, when assessed by the HFMSE and RULM.[82] However, when examined more closely, most of the benefit in the RULM was observed in individuals with type 3 SMA population who are able to sit, but not walk, and although no significant improvement was reported in individuals with type 2 SMA overall, improvements in RULM were observed in individuals with type 2 SMA with residual motor function. Although generally, individuals with more severe disease have worse outcomes, it is clear that SMA type alone as a measure of disease severity is not sufficient to predict the prognosis of individuals with SMA.[83]

In terms of care-related factors, the use of nusinersen treatment is associated with improved motor function outcomes in type 1 SMA[53,68] and types 2 and 3 SMA.[52] The most important modifying factors for nusinersen treatment were age at treatment initiation[52,68] and disease duration,[53] with younger individuals being associated with better motor function outcomes. Further evidence to support this key concept can be taken from recently published studies: Following treatment with the gene therapy onasemnogene abeparvovec, infants with type 1 SMA who were dosed at an earlier age demonstrated larger gains in CHOP-INTEND when compared with those who were older,[84,85] and younger children with type 2 SMA (<6 years) also experienced greater improvement in motor function compared with older children (>6 years) when treated with nusinersen.[86]

In natural history studies, the pattern of disease progression in types 2 and 3 SMA also typically depends on age.[70,71,73,74,87] Younger age groups experience gains in motor function. The steepest declines in motor function occur during adolescence as weight is gained, and contractures and scoliosis develop. As individuals age further (>16 years), declines in motor function plateau as contractures and weight gain stabilize. Recently reported data show that the risk of declining in motor function increases with age and greater baseline motor function.[88]

Factors affecting respiratory measures

Our review found only two high-ROB studies reporting on respiratory outcomes,[26,66] which included no evidence from individuals

treated with DMTs. These studies observed that disease severity and age influence outcomes in SMA. Recent literature in both nusinersen-treated[89] and untreated[90,91] individuals supports these findings.

Limitations

Although we conducted an SLR, our search strategy was restricted by date of publication (January 1, 2000 to April 30, 2019) and to studies published in the English language. As SMA is a rare disease, there is a paucity of data in the SLR from RCTs or high-quality observational studies. Indeed, only three RCTs[49,52,53] were published within the time frame of the SLR.

To assess the quality of the available literature, ROB assessments were conducted on observational studies using the QUIPS tool. Since the QUIPS tool does not provide an overall score, we made an *ad hoc* interpretation of study quality (studies were less reliable if they exhibited four or more domains at a moderate or high ROB). Most of our findings for the observational evidence are informed by univariable analyses, which is suboptimal. Ideally, studies should control for all possible covariate factors when assessing prognostic effect. When studies did not report multivariate analysis control for confounding factors, they were given a moderate/high-ROB score on the QUIPS domain "adjustment for other prognostic factors."

Furthermore, some studies comprised small numbers of patients or subgroups, limiting generalizability and power to detect statistically significant differences. In addition, we did not attempt to calculate statistical significance from graphs or other measures of variability if it was not present in the original study.

The studies in this SLR do not all report the same factors and do not always report the same factors consistently. For example, time to death or permanent mechanical ventilation is a common survival end point in many studies in type 1 SMA. However, permanent ventilation is defined differently in different studies: Finkel, *et al*. 2014 defined permanent ventilation as ≥16 hours per day,[18] whereas in Rudnik-Schoneborn, *et al*.[56] it was for ≥16 hours per day for >14 days. Finkel *et al*.[53] went further and defined permanent assisted ventilation as ≥16 hours per day for >21 continuous days.

It is important to note that many of the prognostic factors analyzed within this study showed some correlation. *SMN2* copy number is associated with SMA type/subtype,[54,55] which is in turn defined clinically by age of symptom onset.[11,12] There is significant correlation between *SMN2* copy number and SMA type: 80% of patients with type 1 SMA possess one or two *SMN2* copies, 82% of patients with type 2 SMA possess three copies, and 96% of type 3 SMA patients possess three or four copies.[6] Although lower *SMN2* copy numbers correlate with lower levels of SMN protein, and lower SMN levels are associated with greater degrees of muscle denervation,[92] the relationship between *SMN2* copy number and disease severity is not absolute; recent studies have shown that other cellular mechanisms may also play a role.[8] This analysis did not consider statistical associations between prognostic factors.

Conclusions

The recent availability of DMTs has revolutionized the management of SMA; treated individuals are now living longer and have improved functional abilities and quality of life. In summary, prognostic factors in patients with SMA include:

- *SMN2* copy number
- baseline motor, bulbar, and respiratory function
- age of symptom onset
- age at study enrollment in natural history studies
- clinical disease severity and SMA type
- the implementation of supportive care

Factors with modifying effects on outcomes of treatment with DMTs include:

- disease duration before DMT initiation
- age at treatment initiation

In treated patients with type 1 SMA[53,68] or types 2 and 3 SMA,[52] disease duration before DMT initiation has been consistently reported as a strong predictor of treatment efficacy. Age at treatment initiation was found to be a prognostic factor in types 1, 2, and 3 SMA, further emphasizing that treatment at a younger age is the most important prognostic factor to contemplate when considering treatment outcomes. In particular, individuals with SMA aged 6–15 years are particularly vulnerable to developing complications, e.g., scoliosis and progressive contractures, which negatively influence functional outcomes. Additional evidence from not-yet published but public data have also been recently reviewed,[93] reinforcing the importance of the effects of these factors on DMT initiation.

Factors beyond treatment that are prognostic of outcomes in SMA include age of symptom onset, supportive therapy, and factors indicative of disease severity, such as the presence of symptoms at birth, and functional status. Disease severity and symptom onset are in turn influenced by a genetic component, principally *SMN2* copy number.

Overall, although *SMN2* was found to have a clear prognostic effect in untreated patients,[54] greater *SMN2* copies were not associated with a significantly better response to treatment[67,68] in infants with type 1 SMA. There is however, published evidence in the literature indicative of better outcomes in patients with three copies of *SMN2* when treated at the presymptomatic stage.[78] In CHERISH,[52] there was some indication of a trend in individuals with types 2 and 3 SMA treated with nusinersen, but due to low copy number diversity, we have no evidence from an RCT to indicate a predictive effect with regard to DMT treatment. Altogether, outside presymptomatic patients, there is no evidence of *SMN2* as a predictor of treatment response. This could be due to the limited amount of long-term data and possible confounding factors in observational studies, such as age at treatment initiation.

The factors discussed in this study, notably age at treatment initiation, the use of supportive therapies and disease duration, should be considered prior to designing or analyzing studies in an SMA population, conducting population matching, or summarizing results from multiple studies on the treatments for SMA. It also prompts the importance of accelerating diagnosis to reduce the disease duration before treatment initiation, which naturally leads to newborn screening programs that have flourished across the world.

Although we have identified key prognostic and predictive factors in this review that determine how well an individual might respond to treatment, there is a need for markers to monitor

responses to ongoing treatment. Although our SLR did not identify any of these studies, in more recent literature there has been a drive to discover new biological markers that can be used to assess how well a patient is progressing.[94-97] For example, plasma phosphorylated neurofilament heavy chain (pNF-H), a marker of axonal damage, is elevated in SMA and reduced in response to nusinersen treatment.[94,97] High levels of pNF-H are associated with an earlier onset of symptoms, later initiation of treatment, and a lower CHOP-INTEND score.[94] Interestingly, most patients with two *SMN2* copies already have signs of disease. Indeed, by the age of 1 month many already exhibit subtle features of disease which can be demonstrated using electrophysiology or by the higher levels of phosphorylated neurofilament.[94]

Future research should be focused on robust statistical methods to adjust for potential confounders (e.g., using multivariable analysis). By synergistically acting, when possible, on prognostic factors, such as the use of supportive therapy, and by treating infants with DMTs as early as possible following a diagnosis, the natural history of this life-threatening disease may be dramatically changed.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

K.G. and M.D. are employees and shareholders of F. Hoffmann-La Roche Ltd. A.K. is an employee of F. Hoffmann-La Roche Ltd. N.H., D.A.S., and R.E. are partners/employees of Visible Analytics Ltd, which conducted this review and received consultancy fees and expenses from F. Hoffmann-La Roche Ltd. A.M. is an employee of Bridge Medical Consulting Ltd, which conducted this review and received consultancy fees and expenses from F. Hoffmann-La Roche Ltd. F.M. reports participation in Scientific Advisory Boards and teaching initiatives for AveXis, Biogen, Roche, and Novartis. He is a member of the Rare Disease Scientific Advisory Board for Pfizer. He is involved as an investigator in clinical trials from AveXis, Biogen, and Roche. In addition, he is the principal investigator of the SMA REACH UK clinical network, partially funded by Biogen and by SMA UK. G.B. is an investigator in spinal muscular atrophy (SMA) trials sponsored by AveXis, Roche, and Novartis. He has received compensation for participation at symposia and Scientific Advisory Boards from Roche and AveXis. L.S. is a principal investigator in SMA studies for Roche, Biogen, and AveXis. He has attended Scientific Advisory Boards of Biogen, Roche, Cytokinetics, and AveXis. He is the coordinating investigator of a study funded by Roche, AveXis, and Biogen.

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