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1 **Spinal muscular atrophy (SMA): from approved therapies to future therapeutic targets**
2 **for personalised medicine**

3

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15

16 **Summary**

17

18 Spinal muscular atrophy (SMA) is a devastating childhood motor neuron disease which, in the
19 most severe cases and when left untreated, leads to death within the first two years of life.
20 Recent therapeutic advances have given hope to families and patients by compensating for the
21 deficiency in survival motor neuron (SMN) protein via gene therapy or other genetic
22 manipulation. However, it is now apparent that none of these therapies will cure SMA alone.
23 In this review we discuss the three currently licensed therapies for SMA, briefly highlighting
24 their respective advantages and disadvantages, before considering alternative approaches to
25 increasing SMN protein levels. We then explore recent pre-clinical research that is identifying
26 and targeting dysregulated pathways secondary to, or independent of, SMN deficiency which
27 may provide adjunctive opportunities for SMA. These additional therapies are likely to be key
28 for the development of treatments that are effective across the lifespan of SMA patients.

29

30 **Keywords:** apoptosis, cytoskeleton, gene therapy, neuroprotection, neuromuscular junction,
31 SMN, splicing modulator, ubiquitination.

32

33 **Introduction**

34

35 Spinal muscular atrophy (SMA), a childhood-onset motor neuron disease, has historically been
36 the most frequent genetic cause of infant mortality,¹ although this is likely to change with the
37 recent therapeutic ‘revolution’. SMA, caused by mutations in the *Survival Motor Neuron 1*
38 (*SMN1*) gene, leads to loss of SMN protein expression. This is partially compensated for by
39 expression from the paralogous gene *SMN2*, which differs from *SMN1* by a single nucleotide
40 polymorphism in exon 7.^{2,3} This base change causes exclusion of exon 7 in nearly 90% of
41 *SMN2* transcripts, and the truncated unstable protein (SMN Δ 7) is quickly degraded.⁴ The SMN
42 protein is ubiquitously expressed and plays a fundamental role in cell homeostasis through
43 multiple functions, which are still not fully understood.⁵ It is involved in various cell
44 mechanisms, such as the assembly of the spliceosomal machinery, endocytosis and protein
45 translation. Due to its diverse roles and ubiquitous expression, loss of SMN can lead to
46 systemic pathology extending beyond the motor neuron, which has to be considered when
47 designing new therapies.⁶

48

49 Within SMA, disease severity varies at least in part according to the number of *SMN2* copies
50 carried by the patient.⁷ In SMA Type 0/1, the most frequent form with one copy of *SMN2*,
51 untreated children will never have the muscular strength to sit unassisted with a life expectancy
52 of around 2 years. As patients carry more *SMN2* copies, the severity of the disease decreases
53 up to Type 4, characterised by an early-adulthood clinical onset and typically a normal life
54 expectancy.¹ However, this natural history of the disease will likely become obsolete as the
55 majority of patients will receive at least one disease-modifying therapy.

56

57 As a rare example of a monogenetic neurodegenerative disease, SMA research has pioneered
58 gene-targeted therapy with the recent approval of three therapies designed to enhance SMN
59 production. Regardless of this transformation in SMA therapy, it has become apparent these
60 novel treatments are still not a cure. In this review, we will first describe the three approved
61 therapies before detailing alternative strategies aimed at increasing SMN levels. Finally, we
62 will discuss other potential targets for drug development.

63

64 **Gene-Targeting SMN Replacement Therapies**

65

66 Currently three treatments, nusinersen (Spinraza®; Biogen), onasemnogene abeparvovec
67 (Zolgensma®; Novartis) and risdiplam (Evrysdi®; Roche), are approved by the U.S. Food and
68 Drug Administration (FDA) and the European Medicines Agency (EMA) (Table 1). Here we
69 will discuss the development of these three drugs in chronological order of licensing, as well
70 as ongoing efforts to improve these strategies.

71

72 *Nusinersen*

73

74 Since manipulation of the splicing pattern of the *SMN2* gene can produce full length, functional
75 SMN protein, strategies that target splicing regulatory elements and boost exon 7 inclusion
76 have shown great success and were the first SMN replacement therapy to achieve regulatory
77 approval. Exclusion of exon 7 from the *SMN2* transcript is regulated by a section of intron 7
78 termed the intronic splicing silencer N1 (ISS-N1), located immediately downstream of the 5'
79 splice site for exon 7, and deletion of the ISS-N1 sequence significantly increased exon 7
80 inclusion.⁸ Manipulation of such regulatory regions by short complementary sequences of
81 synthetic nucleotides is an important therapeutic approach. To determine the most effective

82 target sequence for exon 7 inclusion, multiple antisense oligonucleotides (ASOs) were tested
83 in mice with the sequence shifting along the target by one base at a time.⁹ The strongest
84 sequence (intron 7 position +10-27) was injected as an 18-mer MOE-modified ASO into a mild
85 mouse model at embryonic, neonatal and adult stages. SMN protein levels increased,
86 corresponding to an improvement of the SMA phenotype, and early treatment (embryonic or
87 neonatal) gave a stronger phenotypic correction.¹⁰ The specific ASO-10-27 was able to
88 dramatically improve lifespan, motor function and muscle physiology by either systemic or
89 intracerebroventricular injection in two severe SMA mouse models.^{11,12} Preclinical studies in
90 mouse models and non-human primates showed good distribution of the ASO throughout the
91 spinal cord and dose-dependent effects on SMN expression following a single intrathecal
92 infusion.^{12,13} CNS and systemic delivery of ASOs have to be considered as complementary
93 strategies, since a combined systemic and CNS-directed delivery strategy had the strongest
94 effect on both survival and vascular-related clinical signs in a severe mouse model of SMA.¹⁴
95
96 Based on this preclinical evidence, clinical trials were initiated and subjects found to safely
97 tolerate multiple intrathecal injections in Phase II, with evidence of improvement of motor
98 function and achievement of motor milestones.¹⁵ Successful Phase III data led to FDA and
99 EMA approval in December 2016 and June 2017, respectively, as the first drug to treat 5q
100 SMA Type 1-3 patients (Table 1). Infants treated before 6 months or later, in ENDEAR and
101 CHERISH trials respectively, showed positive results in terms of motor milestones and event-
102 free survival, hence both Phase III trials were terminated at the interim stage to allow all
103 participants to move onto nusinersen in an open-label study (SHINE).¹⁶
104
105 The most recent data from clinical trials for nusinersen provides evidence of long-term safety
106 and efficacy across patient groups, with dramatically improved survival and motor

107 function.^{16,17} After approximately three years of treatment, 100% of SMA Type 2 patients were
108 able to sit unsupported with some able to walk with support, while 76% of SMA Type 3 patients
109 could walk independently.¹⁷ Results also suggest that early treatment may maximise efficacy.
110 Those in the NURTURE clinical trial, which treated infants pre-symptomatically before 6
111 weeks of age for the first dose, had higher motor function at every point of observation after
112 treatment, with the latest data showing 22 out of 25 children able to walk independently and
113 none requiring permanent ventilation.¹⁸ Nusinersen is now available in many countries, with a
114 wider perspective on its efficacy in a range of patient groups,¹⁹⁻²¹ and some evidence that
115 clinical improvement continues after the first year of treatment.²² Finally, a recent study
116 assessed the distribution of ASO and the associated SMN increase in the spinal cord of post-
117 mortem samples from infants treated with nusinersen. This study suggests that there is
118 variability in distribution of ASO within the central nervous system, with a lower concentration
119 in the cranial portion of the spinal cord and in the brain.²³ However, these results need to be
120 put in perspective considering these patients died in infancy and as such were poor responders
121 to the therapy.

122

123 *Onasemnogene Apeparvovec*

124

125 Since the causative factor of SMA is a monogenic defect leading to the loss of SMN protein,
126 this motor neuron disease is a prime candidate for gene replacement therapy. The idea of using
127 viral vectors to deliver exogenous genes to relevant tissues was expanded to neurodegenerative
128 diseases when it was demonstrated that systemic delivery via intravenous injection (IV) of
129 AAV9-based gene transfer can cross the blood brain barrier (BBB)²⁴ and efficiently transduce
130 target cells in the central nervous system (CNS), including motor neurons in the spinal cord in
131 mice and non-human primates.^{24,25} Development of the self-complementary AAV9 (scAAV9)

132 vector further improved the efficiency and speed of gene transcription.²⁶ Using this technique,
133 AAV9-mediated SMN gene expression delivered at postnatal day 1 (P1) significantly
134 improved lifespan and motor symptoms in SMA models.²⁵⁻²⁷ Initial studies also showed that
135 early treatment of SMA mice at P1 generated better outcomes compared to treatment at P5 or
136 P10, highlighting the importance of early intervention in SMA therapy.²⁷

137

138 These successes led to the initiation of a Phase I clinical trial of onasemnogene abeparvovec
139 (previously AVXS-101; onasemnogene hereafter): a recombinant AAV9 viral vector encoding
140 human SMN protein under the control of the cytomegalovirus enhancer/chicken- β -actin hybrid
141 promoter injected intravenously. This first trial treated 15 infants, 3 with low dose and 12 with
142 high dose: all 15 patients survived to 20 months without the need for respiratory support, with
143 11 patients reaching the motor milestone of sitting unassisted and 2 even walking
144 independently.²⁸ This positive Phase I trial and interim data released from the successive Phase
145 II/III trial led to FDA approval in 2019 (Table 1). However, systemic administration by IV of
146 high dose AAV9-SMN was found to cause liver dysfunction in non-human primates.²⁹ This
147 has led to the temporary suspension of an on-going clinical trial (NCT03381729) intending to
148 administer high dose onasemnogene intrathecally, pending further clarification on pre-clinical
149 findings. Nevertheless, in a Phase III clinical trial, 22 patients with symptomatic Type 1 SMA
150 received onasemnogene; out of these, 13 infants achieved independent sitting at 18 months of
151 age. 91% of patients did not require permanent ventilation by the age of 14 months, compared
152 to only 26% of the untreated group.³⁰ Other trials include Phase III STRIVE-EU
153 (NCT03461289) and STRIVE-AP (NCT03837184), which have been assessing efficacy and
154 safety in SMA infants under 6 months of age with 1 or 2 copies of *SMN2*, and SPRINT
155 (NCT03505099) which enrolled SMA infants under 6 weeks old to evaluate the efficacy and
156 safety in pre-symptomatic patients. Liver toxicity remains so far the main adverse event, with

157 the majority of patients receiving onasemnogene also requiring prednisolone treatment to
158 mitigate the hepatotoxicity.³¹

159

160 *Risdiplam*

161

162 Risdiplam (RG7916), a small molecule splice modulator, was approved by the FDA in 2020
163 and the EMA in 2021. Risdiplam acts as an *SMN2* splice modulator, directly binding to *SMN2*
164 pre-mRNA at two sites, an exon enhancer sequence and the 5' splicing site of exon 7,
165 stabilising the ribonucleoprotein complex and competing with hnRNPG binding, thus
166 promoting exon 7 inclusion and full-length SMN protein production.³² Risdiplam was
167 optimised from RG7800, a splice modulator found to increase full length *SMN2* mRNA two-
168 fold in healthy adults and SMA patients.³³ However, development of RG7800 was put on hold
169 due to retinal damage in non-human primates.³⁴ Risdiplam belongs to the SMN-C class of
170 splice modulators: it increased full length SMN protein in both severe and mild mouse models
171 of SMA, with an increase in survival and improvement of motor phenotypes.³⁵

172

173 Oral dosing of risdiplam at 3 mg/kg/day for 7 days in non-human primates showed good
174 biodistribution in relevant tissues,³⁶ while a Phase I trial confirmed good tolerance and no
175 evidence of retinal pathology.³⁷ Recently published results from the Phase II/III FIREFISH
176 study determined improved efficacy at the higher tested dose, for which 7 out of 17 infants
177 were able to sit independently after 12 months of treatment. A follow-up study is ongoing
178 focussing exclusively on the long-term effects of the higher dose.³⁸ Further phase III clinical
179 trials are ongoing, with SUNFISH assessing effectiveness in Type 2/3 patients aged 2 to 25
180 (NCT02908685), JEWELFISH investigating the effects of risdiplam in patients previously
181 treated with other SMA therapies (NCT03032172), and RAINBOWFISH studying risdiplam

182 in pre-symptomatic SMA infants under 6 weeks old at first dose (NCT03779334). Extensive
183 ophthalmologic assessment has also been performed in the patients enrolled in the FIREFISH,
184 SUNFISH, and JEWELFISH clinical trials and did not reveal any retinal toxicity.³⁹

185

186 *Other potential drugs targeting SMN2 splicing*

187 Methods of increasing SMN expression via splice modulation continue to be researched for
188 improved efficacy. Branaplam was identified using a high-throughput screen for *SMN2* exon
189 7 inclusion, and appears to stabilise *SMN2* pre-mRNA with splicing factor complexes.⁴⁰ Daily
190 administration showed a dose-dependent increase of exon 7 inclusion and SMN protein
191 expression in SMA mice with an improvement in body weight and lifespan.⁴⁰ The first in-
192 human Phase I/II trial started in 2015 across trial centres in Europe (NCT02268552) treating
193 SMA patients younger than 6 months old with 2 copies of *SMN2* (Table 1). Interestingly,
194 Novartis has announced a finding of reduced huntingtin mRNA (the mutated protein in
195 Huntington's Disease) in SMA patients treated with branaplam, which has formed the basis of
196 the FDA's Orphan Drug Designation for branaplam in Huntington's disease with a Phase IIb
197 trial planned in 2021.⁴¹

198

199 *In vitro* splicing screens on *SMN2* have identified several novel small molecules which
200 modulate splicing patterns and improve exon 7 inclusion, including the identification of
201 risdiplam and branaplam. Further molecules from the screen initially identifying risdiplam
202 continue to be developed to further enhance *SMN* splicing.⁴³ Another recently discovered small
203 molecule, TEC-1, with a similar chemical structure to risdiplam, has demonstrated increased
204 SMN expression, increased lifespan and improved motor phenotypes in a severe SMA mouse
205 model, with fewer off-target splicing changes than risdiplam.⁴² Further screens have identified
206 that flunarizine, a calcium channel blocker, can alter splicing events in HeLa cells including

207 intron retention in *SMN*.⁴³ This was confirmed in a screen on SMA patient fibroblasts, where
208 flunarizine increased localisation of SMN to Cajal bodies and improved the survival of spinal
209 cord motor neurons from SMA mice.⁴⁴

210

211 Alternatively, whilst nusinersen shows a strong effect on splicing and good biodistribution and
212 pharmacokinetics, it may be possible to improve these outcomes through adjusting the ASO's
213 target or chemistry. As such, optimisation of ASOs targeting *SMN2* splicing continues, with
214 alternative potential target sequences⁴⁵ and improved chemistries, including cell-penetrating
215 peptides.⁴⁶

216

217 *Critical Appraisal of Current Therapies*

218

219 These three licenced therapies have been at the forefront of the therapeutic 'revolution' for
220 SMA. However, there remain some major limitations and concerns. Firstly, nusinersen must
221 be delivered via invasive intrathecal injection multiple times per year. This leads to CNS-
222 specific distribution, meaning that systemic symptoms may not be fully addressed.
223 Onasemnogene is administered intravenously, and so will have systemic distribution, but there
224 remains the issue of bioavailability of the AAV9 serotype and the fact that long-term transgene
225 expression is expected to be limited to post-mitotic cells such as neurons. Conversely,
226 risdiplam is a systemic therapy and is the least invasive licenced treatment with daily oral
227 administration. However, as risdiplam targets the splicing machinery, it may also affect other
228 transcripts leading to unknown off-target side effects. For example, although risdiplam appears
229 to have very high specificity to *SMN2*, the cell division regulator *FOXMI* is also targeted by
230 risdiplam at high concentrations risking oncogenic side effects,³⁴ and so dosage has been
231 strictly monitored in clinical trials. The long-term effects of all three licensed therapies are

232 currently unknown, and may only become apparent many years after onset of treatment. As it
233 stands, through necessity, most available clinical data are through clinical trials funded by
234 pharmaceutical industries, with specific inclusion criteria and short follow-up. Real-world data
235 from a wide spectrum of patients over an extended period of time are slowly becoming
236 available and highlight the shortcomings of current SMN replacement therapies. Treated
237 children remain disabled with complex needs and high level of care requirements.^{19,20,47}
238 Moreover, whilst none of these therapies represents a cure for SMA, each of these therapies
239 carries a high price tag. The one-off onasemnogene injection is priced at \$2.1 million, the most
240 expensive drug in the world, with nusinersen costing \$750,000 in the first year followed by
241 \$375,000 annually thereafter, and risdiplam priced at up to \$340,000 per year.⁴⁸ These costs
242 can clearly lead to major issues for patients and their families, as well as healthcare providers
243 (Table 1). Furthermore, although onasemnogene is advertised as a single injection therapy, it
244 remains unclear whether the treatment will be lifelong or if additional therapies will be
245 required. Currently, no clinical guidelines are available to help clinicians and families choose
246 one therapy over another for a specific patient. Direct comparison of these therapies is made
247 difficult due to different inclusion criteria, assessment and outcome measures across clinical
248 trials. This is a critical issue that will have to be addressed through unbiased clinical trials
249 specifically designed for comparison.

250

251 **Wider Therapeutic Strategies - Beyond the Gene Targeting Drugs**

252

253 As mentioned above, these currently licensed therapies have changed the phenotype of SMA
254 patients. However, the clinical trials and real-world data highlight the need for adjunctive
255 therapies in order to improve the quality of life of these patients. Research has therefore
256 focussed on addressing the systemic consequences of SMN loss. As such, the potential targets

257 are not specific to SMA, but rather fit in a wider strategy for treatment of neuromuscular
258 disorders. A drug that is already approved or in clinical trials for one condition may also be
259 advantageous in SMA, in combination with SMN replacement therapies or alone. This
260 repurposing approach could result in a faster path to the patient due to already available safety
261 profiles. In the following section, we will discuss these broader strategies and highlight those
262 that are already in the clinic for various conditions in Table 2.

263

264 *Non-Specific Therapies Increasing SMN Levels*

265

266 Prior to the development of gene-targeting SMN replacement therapies, multiple approaches
267 have been sought to increase SMN levels using drugs not specifically targeting the SMN gene.
268 Although in the current landscape these drugs may seem redundant, they may be considered as
269 an additional therapy to further enhance SMN expression.

270

271 HDAC Inhibitors

272

273 Histone deacetylase (HDAC) inhibitors have been investigated in SMA models since the early
274 discovery that histone acetylation controls SMN expression.⁴⁹⁻⁵¹ Multiple HDAC inhibitors are
275 either currently licenced or in clinical trials for cancer treatments (Table 2), hence make
276 attractive therapies for alternative indications due to their known safety profiles. Screening
277 HDAC inhibitors in SMA patient-derived neuronal cells showed that targeting class I HDACs
278 in particular could boost SMN expression.⁵²

279

280 Valproic acid is a classic class I HDAC inhibitor that has shown beneficial effects in mouse
281 models of SMA⁵³ and patient fibroblasts⁵⁰ so was quickly moved in to clinical trials. A

282 systematic review and meta-analysis of valproic acid clinical trials up to 2017 suggested an
283 overall beneficial effect in motor function, but little evidence of change in survival.⁵⁴ Another
284 class I HDAC inhibitor, phenylbutyrate, showed promising levels of SMN expression elevation
285 in patient fibroblasts,⁵¹ but showed extremely variable outcomes in patients⁵⁵ and the clinical
286 trial was prematurely terminated (NCT00439569). Other small molecules with HDAC
287 inhibitor properties include suberoylanilide hydroxamic acid (SAHA⁵⁶), trichostatin A⁵⁷, and
288 resveratrol.⁵⁸ However, although these molecules showed success in laboratory models of
289 SMA, they have not been progressed to the clinic.

290

291 HDAC inhibitors alone cannot provide a therapeutic benefit to the same levels as SMN
292 replacement.⁵⁴ However, they may provide additional neuroprotective support in combination
293 with other SMN targeting therapies ('SMN+' therapies⁵⁹). This idea is exemplified in a recent
294 paper showing the benefits of combinatorial therapy between the HDAC inhibitor LBH589
295 (Panobinostat) and low doses of Spinraza-like ASOs.⁶⁰

296

297 R-loops

298

299 During transcription, the double-stranded DNA structure is broken to allow new RNA
300 transcripts to be created, and the DNA-RNA structure with the free non-coding DNA strand is
301 called an R-loop. This physiological process is tightly modulated by nuclear factors and
302 DNA/RNA binding proteins. However, R-loops can be formed, or not resolved properly, under
303 pathological conditions, thereby disrupting physiological processes and leaving the single-
304 stranded DNA more susceptible to degradation. Suggested mechanisms for pathological R-
305 loop formation have been mutations in, or up/downregulation of, factors controlling R-loop
306 generation or particularly G-rich DNA sequences. Since R-loop formation can be governed by

307 splicing factors, and SMN has a key role in assembly of the spliceosomal small nuclear
308 ribonucleoproteins so its loss leads to widespread splicing defects, these two pathologies may
309 be linked. In cell culture, *SMN1* knockdown led to increased numbers of R-loops over retained
310 introns, and overexpression of RNase H1 (a factor that helps resolve R-loops) prevented DNA
311 damage.⁶¹ Pathological R-loop formation is therefore a potential therapeutic target, but using
312 DNA-binding molecules as therapies comes with an obvious mutagenic risk. Senataxin on the
313 other hand is a DNA repair factor that co-localises with SMN in Cajal bodies and has a
314 decreased expression in SMA models. Overexpression of Senataxin in SMA mouse spinal cord
315 motor neurons reduced R-loop formation and DNA damage.⁶² Another nuclear factor, zinc
316 finger protein ZPR1, was also found to have reduced expression in SMA models.⁶³ Its
317 overexpression doubled the survival of an intermediate mouse model, improved their righting
318 reflexes, and increased motor neuron survival and muscle fibre diameter.⁶³ ZPR1
319 overexpression was shown to increase expression of *Smn* itself, but could clearly have more
320 global protective pathways as well.

321

322 Stabilising the SMN protein

323

324 Preventing the degradation of SMN Δ 7, the product of *SMN2*, thus allowing even low-level
325 expression to have a more pronounced effect on intracellular pathways, could be a therapeutic
326 strategy. Indoprofen is an NSAID that was identified in an *SMN2*-luciferase screen to increase
327 SMN protein levels in patient fibroblasts.⁶⁴ This screen was also used to identify other
328 compounds that increase SMN expression *in vitro* and *in vivo*.⁶⁵ A novel aminoglycoside,
329 TC007, was found to act as a read-through compound for exon 8 of *SMN2*, increasing the
330 number of nuclear gems in patient fibroblasts⁶⁶ and can slightly increase survival of the severe
331 SMA mouse model.⁶⁷ Other read-through compounds such as azithromycin have shown some

332 efficacy in mouse models.⁶⁸ Finally, preventing degradation of SMN using the proteasome
333 inhibitor bortezomib improved survival and motor outcomes in SMA mouse models.⁶⁹
334 However, none of these approaches have yet reached clinical trials for SMA (Table 2).

335

336 *Neuroprotection*

337

338 Since motor neurons are the most severely affected cell type in SMA, it follows that
339 neuroprotective strategies targeted at this neuronal population may be effective, in particular
340 when employed in combination with SMN-restoring therapies (SMN+’ therapies⁵⁹).

341

342 Bioenergetics

343

344 Neurons and muscle, the major tissue types affected in SMA, have particularly high energy
345 demands and so targeting energy pathways may be neuroprotective and therapeutic in SMA.
346 The glycolytic enzyme PGK1 was found to be dysregulated in SMA mouse models, and
347 increasing its activity pharmacologically with terazosin or its expression genetically could
348 ameliorate motor axon phenotypes in SMA zebrafish models.⁷⁰ Alternatively, olesoxime, a
349 mitochondria-targeting therapy, was originally shown to promote cell survival under stressed
350 conditions *in vitro* likely via modulation of mitochondrial membrane permeability.⁷¹ As such,
351 olesoxime could be broadly applicable across neurodegenerative diseases, and was found to
352 triple the lifespan of the SMA severe mouse model.⁷¹ Olesoxime showed a good safety profile
353 in Type 2/3 patients as well as an improvement in motor function (Table 2).⁷² It was therefore
354 moved on to Phase III clinical trials, but the trial was cancelled by Roche amidst the progression
355 of nusinersen, onasemnogene and risdiplam due to reported issues with dosage and production.

356 However, due to the current pressing demand for combinatorial treatments, olesoxime may
357 return as a subject for future research.⁷³

358

359 Excitotoxicity

360

361 An early strategy in SMA research was to test efficacious drugs from other neurodegenerative
362 models, particularly ALS (Table 2), and so drugs that target excitotoxicity have been tested in
363 SMA. Riluzole was used in a small preliminary Phase I trial based on its modest effects in
364 ALS, which gave a suggestion of an effect with a sample size of 7.⁷⁴ A Phase II/III trial to
365 evaluate efficacy of riluzole in SMA patients was completed in 2013, but no results have been
366 posted (NCT00774423). Gabapentin is another drug targeting excitotoxicity that was tested in
367 Type 2/3 SMA patients based on its effect in ALS, showing some effect on motor function in
368 one study⁷⁵ but none in another.⁷⁶ Edaravone, a drug approved for ALS in the US and Japan,
369 showed some promise in SMA patient-derived iPSC-motor neurons,⁷⁷ but has not yet been
370 taken further. The same group found levetiracetam, an anti-epileptic drug, to have therapeutic
371 potential in their *in vitro* SMA model.⁷⁸

372

373 *Muscle-targeting Therapies*

374

375 Intrinsic and denervation-induced muscle pathology plays an important role in SMA. This was
376 recently confirmed by an elegant study where selective depletion of SMN in skeletal muscle
377 of mice was enough to induce muscular and NMJ pathology.⁷⁹ It has also been hypothesised
378 that improving muscle pathology could lead to preservation of proprioceptive synapses onto
379 motor neurons that are lost in SMA.⁸⁰ Therefore, muscle is considered a promising therapeutic
380 target via numerous strategies: myostatin inhibition; activating fast troponin complexes;

381 modulating metabolic and ergogenic pathways; and enhancing mitochondrial function (Figure
382 1).

383

384 Myostatin is a negative regulator of muscle growth and inhibition of the myostatin signalling
385 pathway has shown promising results, especially in less severe models of SMA⁸¹ or in addition
386 to SMN restoring therapies.^{80,82} Numerous inhibitory strategies have been trialled, such as
387 antibodies directed against myostatin (or its precursors),⁸² against the myostatin receptor
388 (ActRIIB),⁸¹ or using follistatin⁸³ as an endogenous antagonist of myostatin. Due to high
389 homology between myostatin and other growth factors of the TGF β family, high specificity
390 against myostatin is necessary to avoid previously observed side-effects of the first clinically
391 tested compounds in Duchenne muscular dystrophy (DMD) (gingival bleeding, telangiectasias,
392 and hormonal level changes).⁸⁴ A selective myostatin inhibitor (SRK-015) has shown promise
393 in SMA mice⁸² and has been tested in Type 2/3 SMA patients (NCT03921528; Table 2).
394 Interim results revealed an increase in motor function in patients receiving the high dose, with
395 no significant adverse effects.⁸⁵ An ActRIIA/B ligand trap is also currently in Phase I
396 (BIIB110).

397

398 Fast skeletal muscle troponin activators prolong calcium binding to the troponin complex of
399 fast skeletal muscle, hence increasing muscle contractility and reducing the energetic cost of
400 contraction.⁸⁶ This target is the basis of numerous small molecules being developed by
401 Cytokinetics. Unfortunately, the results of a Phase II study in SMA Type 2-4 patients treated
402 with Reldesemtiv (CK-2127107) were not promising; of the 10 primary outcomes, only an
403 improvement in maximum expiratory pressure was observed (NCT02644668).

404

405 Another approach is modulation of systemic anabolic pathways as an ergogenic strategy. For
406 example, IGF-1 has an anabolic effect on various tissues including the CNS and muscle and
407 there is some evidence that enhancing IGF-1 signalling could be beneficial in SMA.⁸⁷
408 However, a trial in SMA Type 2/3 patients treated with synthetic somatotropin (growth
409 hormone, leading to increased IGF1 expression) did not show any improvement in muscle
410 function.⁸⁸ Similarly, the transcription factor KLF15, regulating metabolic and ergogenic
411 muscular pathways, was downregulated in SMA mouse models although the first preclinical
412 trials only showed limited effect (Table 2).⁸⁹

413

414 *Targeting the Neuromuscular Junction (NMJ)*

415

416 SMA is associated with an impairment of NMJ development, maturation and function, which
417 contributes to muscle weakness and fatigability.⁵⁹ In this section, we focus on therapies
418 specifically targeting the NMJ. Other therapeutic strategies can also improve NMJ pathology
419 e.g. by enhancing cytoskeleton dynamics (see Cytoskeleton section below and Figure 1).

420

421 The agrin/MuSK signalling pathway, which plays a key role in the formation and maturation
422 of the NMJ, is dysregulated in SMA. Overexpression of agrin, or its downstream mediators
423 such as DOK7, improves NMJ structure and reduces disease severity in SMA mice.^{90,91}
424 Subcutaneous administration of NT-1654, the active portion of agrin, also delayed SMA mouse
425 disease progression.⁹²

426

427 Increased fatigability is a major symptom in SMA Type 2/3 patients and repeated nerve
428 conduction studies show a decrement, confirming that NMJ dysfunction could be playing a key
429 role in fatigability.⁹³ Consequently, a Phase II clinical trial⁹⁴ is currently assessing the effects

430 of pyridostigmine, an acetylcholinesterase inhibitor routinely prescribed for myasthenia gravis
431 to slow degradation of acetylcholine within the synaptic cleft, increasing the cholinergic
432 transmission efficiency. Preliminary reports suggested that pyridostigmine reduces fatigability
433 in patients and should be considered as a possible adjunctive therapy (Table 2).⁹⁵

434

435 Salbutamol, a β 2-adrenoreceptor agonist, modestly improves muscle strength in patients by
436 increasing levels of *SMN2* full-length mRNA and protein⁹⁶ and possibly through stabilisation
437 of acetylcholine receptor clusters at the NMJ.⁹⁷ Although small clinical studies suggest that it
438 could help maintaining motor function⁹⁸ or improve respiratory function⁹⁹ of SMA Type 2
439 patients, large scale, placebo-controlled studies are lacking.

440

441 Improving neurotransmission at the NMJ is another possible therapeutic strategy. By blocking
442 potassium channels, 4-aminopyridine prolongs the presynaptic action potential and increases
443 acetylcholine release at the NMJ, therefore improving neurotransmission and motor function
444 in a *Drosophila* model of SMA.¹⁰⁰ However, a recent pilot study could not detect any
445 improvement on locomotion in a group of ambulatory adult SMA patients.¹⁰¹ Amifampridine
446 (3,4-DAP), another aminopyridine approved for the treatment of Lambert-Eaton myasthenic
447 syndrome, has also been evaluated in ambulatory, SMA Type 3 patients (NCT03781479).
448 Enrolment is complete but results are yet to be released.

449

450 Finally, calcium signalling is also altered in nerve terminals of SMA mice and is associated
451 with decreased neurotransmitter release.¹⁰² By slowing down the closure of the voltage gated
452 calcium channels, R-roscovitine increases presynaptic calcium influx.¹⁰³ This resulted in
453 increased survival and improvement of NMJ morphology in an SMA mouse model.

454

455 *Targeting the cytoskeleton*

456

457 The cytoskeleton plays a key role in maintaining compartmentalisation and polarisation of
458 neurons. In SMA, aberrant upregulation of the RhoA/Rho kinase (ROCK) pathway leads to
459 disruption of actin pathways, affecting neuronal growth, differentiation and regeneration.¹⁰⁴
460 Pharmacological inhibition of the ROCK pathway by Y-27632¹⁰⁵ and the FDA-approved drug
461 Fasudil¹⁰⁶ improved survival, NMJ maturation and muscle development in an intermediate
462 mouse model of SMA. One of the downstream mediators of the ROCK pathway, PTEN, could
463 be another potential target.¹⁰⁷ Microtubule dynamics are also affected in SMA as stathmin-1, a
464 microtubule-destabilizing protein, is a disease modifier and overexpression improved survival,
465 motor function and NMJ pathology in an SMA mouse model.¹⁰⁸

466

467 *Targeting endocytosis*

468

469 There is a growing body of evidence suggesting that perturbation of endocytosis plays an
470 important role in SMA pathophysiology.^{109,110} Plastin 3 (PLS3), an actin bundling protein,¹¹¹
471 and neurocalcin delta (NCALD), a neuronal calcium sensor and negative regulator of
472 endocytosis,¹¹⁰ have been identified as two strong SMN-independent protective modifiers in
473 SMA patients. Overexpressing PLS3 partially rescued motor neuron pathology, especially
474 NMJ structure and function, over a wide range of animal models of SMA,^{109,111} although it was
475 insufficient to reverse the pathology in a severe mouse model.¹¹² Similar therapeutic benefits
476 were obtained by decreasing levels of NCALD.¹¹³ Furthermore, PLS3 interacts with coronin
477 1C – an actin bundling protein; with calcineurin-like EF-hand protein 1 (CHP1) – a calcium
478 sensor and calcineurin inhibitor;¹¹⁴ and with members of the hnRNP F/H family of proteins.¹¹⁵
479 Modulation of their respective expression improved impaired endocytic pathways and

480 neuromuscular pathology in SMA models. Considering the overall consistent effect of their
481 modulation on SMA animal models – especially the less severe ones – these represent a
482 particularly attractive target for combinatorial therapy.

483

484 *Cell Death Mechanisms*

485

486 Autophagy, Ubiquitin Homeostasis and Apoptosis

487

488 Core pathways in cell homeostasis, including autophagy, ubiquitin homeostasis and apoptotic
489 pathways, have been linked to neurodegeneration in SMA. Autophagy is a finely tuned system
490 as either excessive or insufficient activity can be pathological. In SMA models, autophagosome
491 numbers are increased.^{116,117} However, it remains unclear whether autophagic flux is increased
492 or decreased.^{116,118} Further studies are warranted as autophagy could represent a therapeutic
493 target. Indeed, administration of 3-methyladenine (an autophagic inhibitor) delayed motor
494 neuron degeneration and subtly increased the lifespan in a severe mouse model.¹¹⁷
495 Additionally, calpains, a calcium-dependent family of proteases, regulate numerous cellular
496 processes including autophagy. Evidence suggests that calpains are overactivated in SMA and
497 inhibition of calpain with calpeptin significantly increased survival and motor activity in SMA
498 mouse models.¹¹⁹

499

500 The other key mechanism for protein degradation is the ubiquitin pathway. Marked
501 dysregulation of this pathway has been shown in SMA and mutations within one of the only
502 two known E1 ubiquitin-activating enzymes, UBA1, is enough to induce X-linked SMA, a rare
503 disorder with similar clinical symptoms to SMA but not associated with *SMN* mutations.¹²⁰
504 Disrupted ubiquitination has been highlighted as a key driver of SMA pathophysiology. A

505 decrease in UBA1 activity is consistently observed across SMA models^{121,122} and restoration
506 of UBA1 activity markedly ameliorated the phenotype of zebrafish and mouse models of
507 SMA.¹²¹ This pathway therefore represents a powerful SMN-independent therapeutic target,
508 but future research identifying small molecules that can stabilise or activate UBA1 will be
509 required to facilitate development as an adjunctive therapy. Moreover, SMN itself is degraded
510 via the ubiquitin system and pharmacological inhibition of SMN ubiquitination by ML372, an
511 E3 inhibitor, increased SMN half-life and thus the lifespan of a severe SMA mouse model.¹²³

512

513 The JNK signalling pathway plays a pivotal role in neuronal apoptosis and is a therapeutic
514 target for multiple neurodegenerative disorders.¹²⁴ The JNK pathway has been shown to be
515 activated in spinal cord of SMA mice and patients¹²⁵ although a more recent study disputed
516 these findings.¹²⁶ Nevertheless, the fact that genetic¹²⁵ and pharmacological inhibition¹²⁷ of the
517 JNK pathway resulted in improved lifespan and motor function would suggest that this may be
518 a therapeutically-relevant pathway in SMA.

519

520 p53-Cell Death Pathway

521

522 Loss of SMN activates the tumour suppressor p53.¹²⁸ The p53 pathway therefore presents a
523 potentially attractive therapeutic target, since it is reported to drive motor neuron cell death in
524 a severe mouse model of SMA.¹²⁹ Loss of SMN may lead to downstream reduction of the ER-
525 localised transmembrane protein Stasimon, thus activating p53.¹²⁸ This same study showed that
526 overexpression of Stasimon was sufficient to block motor neuron degeneration, as mice
527 overexpressing Stasimon showed improved motor function and increased motor neuron
528 numbers.¹²⁸ However, the extent to which this rescue is truly SMN independent is unclear,
529 since further analysis of the subset of mice that showed improved motor function also found

530 twice the levels of full length *SMN2* RNA transcripts compared to the non-responding group.
531 ¹²⁸ Decreased Stasimon expression in SMA models is thought to be due to loss of SMN-
532 mediated U12 intron splicing, and delivery of minor snRNA genes to boost this splicing
533 pathway improved survival and motor function in SMA mice.¹³⁰ This pathway could also be
534 targeted therapeutically via inhibition of p38 using MW150, therefore preventing
535 phosphorylation and activation of p53.¹²⁸ The p38 pathway is also the proposed mechanism of
536 action for celecoxib, an NSAID shown to extend the lifespan of an intermediate mouse
537 model¹³¹ which reached Phase II clinical trials but was recently prematurely terminated with
538 results yet to be published (NCT02876094).

539

540 *Other Potential Therapeutic Targets*

541

542 Neurite Outgrowth

543 The transmembrane protein chondrolectin (*Chodl*), involved in axonal guidance, neurite
544 outgrowth and synaptogenesis, is dysregulated in SMA.^{132,133} Overexpression of *Chodl* rescued
545 motor neuron pathology in a zebrafish model.¹³² However, further experiments, especially in
546 mammalian models of SMA, are required to confirm the therapeutic potential of this approach.
547 The cyclin-dependent kinase 5 (*CDK5*), involved in neuronal architecture maintenance, neurite
548 outgrowth and synaptic plasticity, is overactivated over a wide range of neurodegenerative
549 disorders, including SMA. Therefore, pharmacological inhibitors of *CDK5* could be
550 particularly attractive.¹³⁴ In SMA and other neurodegenerative disorders, *CDK5* hyperactivity
551 leads to tau hyperphosphorylation. The fact that knocking-out tau could ameliorate motor
552 neuron degeneration and synaptic stripping in an SMA mouse model further emphasize the
553 potential role played by *CDK5* and tau in SMA pathophysiology.¹³⁵

554

555 miRNAs

556

557 Micro-RNAs (miRNAs) are regulatory RNA molecules with diverse and interacting roles in
558 the regulation of the cell's internal environment, and can be either blocked or overexpressed to
559 drive a particular pathway. These strategies are being explored across neurodegenerative
560 diseases. As one example, miR-206 drives regenerative pathways at the NMJ in motor neuron
561 disorders and is upregulated at late stages of disease in SMA mouse models as a pro-survival
562 mechanism, but not sufficiently to rescue motor neuron degeneration.¹³⁶ Overexpression of
563 miR-206 extended the lifespan of SMA mice and improved motor performance, suggesting a
564 possible therapeutic option.¹³⁷ miR-23a is another potential therapeutic target, since it was
565 found to be downregulated in SMA iPSC-derived motor neurons and overexpression increased
566 the lifespan of SMA mice.¹³⁸

567

568 Lifestyle Changes

569

570 Metabolic dysregulation is common across SMA mouse models and patient groups,
571 incorporating dysregulation of lipids, amino acids and glucose.¹³⁹ As such, modulation of diet
572 and/or exercise may be of significant therapeutic benefit. Thus, in a mouse model of mild SMA
573 both high-intensity swimming and low-intensity running showed benefits in terms of both lipid
574 and glucose metabolism.¹⁴⁰ The therapeutic benefits of exercise would appear be most
575 applicable to older SMA patients with less severe forms of the disease, or patients with
576 extended survival resulting from treatment with SMN replacement therapies. In particular,
577 lifestyle changes offer a relatively easy (and cheap) way to deliver SMN+ combinatorial
578 therapies.

579

580 **Future Perspectives**

581

582 Over the last few years, the field of SMA research has been revolutionised. Thanks to the
583 development of ground-breaking SMN replacement strategies, there are finally good
584 therapeutic options for families with children diagnosed with SMA, albeit at an extremely high
585 cost.⁴⁸ These therapies are completely changing the phenotype of the treated patients, who will
586 no longer follow the natural history of SMA. With a prolonged lifespan and improved
587 neuromuscular function, non-CNS symptoms could become more of a concern in those treated
588 with CNS-targeting therapies.⁶ However, regardless of the pioneering nature of these therapies,
589 it is becoming obvious with hindsight and long-term follow-up of the first treated patients that
590 SMN protein replacement is not a cure. The timing of treatment is critical, with early treatment
591 having much better prospects,¹⁴¹ but there also appears to be a subsection of patients who do
592 not respond.¹⁶ Several factors may contribute to this variable response, including genetic
593 factors beyond *SMN1* and *SMN2*, environmental factors, or access to and quality of medical
594 care. Some patients have already resorted to combining the various available SMN replacement
595 therapies, with no evidence yet for additional benefit, although clinical trials are ongoing and
596 recruiting at the time of writing.

597

598 It is now well-acknowledged that to improve the chances of a good response to SMN
599 replacement therapy, treatment must be initiated as early in life as possible. The gold standard
600 for SMA treatment should therefore involve neonatal genetic screening, as currently practiced
601 in limited countries in the world. There has even been recent evidence for developmental
602 pathology *in utero* in SMA mouse models,¹⁴² further highlighting that early treatment is key.
603 The SMA field has been captivated over recent years with the development of these SMN
604 replacement therapies. After a few years of clinical experience, we have seen how life-

605 changing these drugs can be, but we are also aware of their limitations, especially for those
606 patients diagnosed later in life or suffering from a milder form of SMA. This calls for SMN+
607 strategies that include SMN-independent therapies, such as those described in this review.
608 Several of these targets, due to their broadly neuroprotective actions, could also be of benefit
609 to other neurodegenerative disorders, particularly other motor neuron diseases. This cross-
610 disease approach, particularly focussing on repurposing drugs with known safety profiles
611 (Table 2), could drive therapies faster along the path to the patient.

612

613 Due to the ubiquitous roles of SMN in the cell,⁵ it is not surprising that numerous therapeutic
614 targets are being identified. Animal experiments have shown that a lot of these targets may
615 have more efficacy in milder forms of the disease. This not only advocates for their use as a
616 combinatorial therapy but also calls for a reassessment of previous targets whose effects may
617 have been overlooked when they could not overcome the particularly severe phenotype alone.
618 Currently the therapeutic potential of most of these targets have been evaluated by genetic
619 manipulation in animal models and future emphasis should be placed on bridging the gap
620 between target discovery and small molecule development.

621

622 **Conclusion**

623

624 The three currently approved drugs for SMA replacement therapy have given life-changing
625 treatment options to SMA patients and their families for the first time. All three treatments
626 extend life expectancy and allow patients to reach motor milestones that would previously have
627 been unachievable. However, the limitations of these therapies are now apparent, opening the
628 road for development of wider targets beyond SMN replacement. Before the efficacy of gene
629 replacement and/or splice modulation was confirmed in clinical trials, alternative SMN-

630 dependent and SMN-independent strategies were investigated. These may still play an
631 important role in SMA therapy, allowing both combinatorial and systemic approaches to be
632 developed. Such approaches targeting pathophysiological events occurring in SMA may also
633 have benefits for other neurodegenerative and neuromuscular diseases. The development of
634 SMN replacement therapies is not the end of the road for SMA therapy development. On the
635 contrary, they have opened a new world of possibilities.

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638

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642

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644

645 **Competing Interests:** THG has served on SMA advisory boards for Roche.

646

647

648 **Figure 1: Schematic of the main SMN-independent potential therapeutic targets.**
649 *Due to the diverse cellular roles and ubiquitous expression of SMN, SMN-deficiency leads to*
650 *changes in numerous cellular processes and organs, which have been identified as possible*
651 *therapeutic targets. For clarity, we classified these targets into cellular pathway degradation,*
652 *neuroprotection, cytoskeleton, muscle and neuromuscular junction, but some therapies may*
653 *span over multiple targets.*
654
655
656

Drug / Company	Mechanism of action	Stage of development	Route of administration and protocol	Population targeted by the licence	Cost ^{48*}	Other comments
<i>Approved Therapies:</i>						
Nusinersen (Spinraza®) / Biogen	Splicing modifier of <i>SMN2</i> (antisense oligonucleotide)	Approved by the FDA (Dec. 2016) and the EMA (May 2017).	Intrathecal administration: 3 loading doses at 14-day interval, 4th loading dose 30 days after the 3rd dose, and maintenance dose every 4 months thereafter	All ages and all types of SMA	Up to \$125,000 per dose. Drug cost for the first year: \$750,000; then \$375,000 annually. Rebates have been obtained by some countries and organisations but in most cases, this is not transparent	<ul style="list-style-type: none"> • Long-term efficacy and side-effects unclear • Intrathecal administration difficult/impossible for patients who had surgeries for scoliosis, making nusinersen not an option for these patients • Optimum dosing and protocol have been underexplored and ongoing trials are evaluating the potential of higher dosing (NCT04089566) • Approved for use in adults despite the lack of clinical trials on adults at the time of approval and unknowns regarding the dose. First studies suggests it could be promising in some patients^{143,144} but further trials are required • Targets the central nervous system
Onasemnogene abeparvovec-xioi	Replacement of <i>SMN1</i> gene	Approved by the FDA (May 2019)	Intravenous injection (single dose)	FDA: Treatment of paediatric patients less than 2 years of age with	\$2,125,000 (single injection)	<ul style="list-style-type: none"> • Limited experience in patients over 2 year-old

(Zolgensma®) / Novartis		and the EMA (conditional approval May 2020).		spinal muscular atrophy (SMA) with bi-allelic mutations in the <i>survival motor neuron 1 (SMN1)</i> gene. EMA: patients with 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the <i>SMN1</i> gene and a clinical diagnosis of SMA Type 1, or patients with 5q SMA with a bi-allelic mutation in the <i>SMN1</i> gene and up to 3 copies of the <i>SMN2</i> gene.		<ul style="list-style-type: none"> • Requires an immunomodulatory³¹ regimen with prednisolone prior and after intravenous infusion (for at least 2 months including tapering period) to decrease the response to the adeno-associated viral vector serotype 9 (AAV9) capsid • Long-term efficacy and safety unclear • Thought to remain primarily in post-mitotic cells (e.g. neurons), hence a not true systemic effect • Irreversible treatment
Risdiplam (Evrysdi®) / Roche	Splicing modifier of <i>SMN2</i> (small molecule)	Approved by the FDA (Aug 2020) ¹⁴⁵ and the EMA (Mar 2021) ¹⁴⁶	Oral - once daily	Patients 2 months of age and older	Up to \$340,000 a year (cheaper in younger patient as dosing is weight-related)	<ul style="list-style-type: none"> • Long-term efficacy and safety unclear • Oral administration allows for systemic treatment
<i>In Clinical Development:</i>						
Branaplam / Novartis	Splicing modifier of <i>SMN2</i> (small molecule)	Still under development. Enrolment in Phase I and II completed but results	Oral. Still under development (weekly administration)	Not yet applicable Current clinical trial focused on Type 1 with 2 <i>SMN2</i> copy numbers	Not yet applicable	<ul style="list-style-type: none"> • Initial enrolment had been halted in 2016 due to safety concerns • Resumed in end 2017 after amendments to protocol (NCT02268552)

		have not yet been released ¹⁴⁷	in the clinical trial)			<ul style="list-style-type: none"> • Oral administration would allow for systemic treatment
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657

658 ***Table 1: Summary of selected SMN-targeted therapies approved or in clinical development.***

659 * the costs given are indicative and do not include hospitalisation and procedure fees.

660

Name of molecule	Mechanism of action for treatment of SMA	Licensed (indication)	Clinical trials in SMA				Clinical trials in other neurological/neuromuscular diseases
			Trial Number	Phase	Patient Group	Results	
<i>NON-SPECIFIC THERAPIES INCREASING SMN LEVELS</i>							
Flunarizine	Splice modulation	Y (not in all countries - migraines)	N/A				N/A
Valproic Acid	HDAC Inhibitor - Increased SMN expression	Y (bipolar disorder, migraine prophylaxis, epilepsy)	Meta-analysis of clinical trials up to 2017 indicated an improvement in motor function but not survival ⁵⁴				ALS - NCT00136110 (Phase III): completed, no results posted, NCT03204500 (Phase II): completed, no results posted
Sodium Phenylbutyrate	HDAC Inhibitor - Increased SMN expression	Y (urea cycling disorders)	NCT00528268	I/II	Presymp. Type 1/2	Completed 2015 Secondary outcomes of improved motor function and body mass not reported Terminated due to poor compliance Terminated due to slow recruitment	ALS - NCT03127514 (Phase II): part of combination treatment PB-TURSO, results showing improvement in motor function and survival ¹⁴⁸ IBM - NCT04421677 (Phase I): ongoing
			NCT00439569		Type 2/3		
			NCT00439218	I/II	Type 1		
Suberoylanilide hydroxamic acid (SAHA; vorinostat)	HDAC Inhibitor - Increased SMN expression	Y (lymphoma)	N/A				AD - NCT03056495 (Phase I): recruiting
Resveratrol	HDAC Inhibitor - Increased SMN expression	Y (dietary supplement)	N/A				ALS - NCT04654689 (Phase II): not yet recruiting Muscular dystrophies – (Phase IIa): improvement in muscle function ¹⁴⁹
LBH589 (Panobinostat)	HDAC Inhibitor - Increased SMN expression	Y (myeloma)	N/A				N/A
Azithromycin	Increased SMN expression	Y (antibiotic)	N/A				N/A
Bortezomib	Increased SMN expression	Y (myeloma/ lymphoma)	N/A				MG - NCT02102594 (Phase II): terminated due to low recruitment
<i>NEUROPROTECTION</i>							
Terazosin	PGK1 activation	Y (hypertension)	N/A				N/A

Olesoxime	Mitochondrial protection	N	2006-006845-14 NCT01302600	Ib II	Type 2/3 Type 2/3	Well-tolerated Well-tolerated, no change in motor function Long-term motor decline, matched to natural history control data	ALS - NCT00868166; NCT01285583 (Phase II/III): add-on to riluzole, with no effect on survival or motor function MS - NCT01808885 (Phase I): no results posted
Riluzole	Glutamate receptor antagonist	Y (ALS)	NCT00774423	II/III	Type 2/3	No results posted	ALS – Cochrane systematic review based on 4 clinical trials suggests that riluzole increases life expectancy by 2-3 months ¹⁵⁰
Gabapentin	VGCC inhibitor	Y (focal seizures and others including muscular symptoms in ALS)	- -	II/III II/III	Type 2/3 Type 2/3	No effect on any outcome measure ⁷⁶ Improvement in limb strength tests but no change in respiratory tests ⁷⁵	N/A
Edaravone	Antioxidant	Y (ALS – USA and Japan only)	N/A				ALS – NCT00330681 (Phase III): no significant functional difference. Nevertheless post-hoc analysis suggested it could be effective in patients with shorter disease duration and milder symptoms. NCT01492686 (Phase III): restricted to patients with shorter disease duration and milder symptoms: slower functional decline in treated patients.
Levetiracetam	Anti-epileptic	Y (epilepsy)	N/A				ALS - NCT00324454 (Phase II): no results posted AD - NCT03489044 (Phase II): no results posted
<i>MUSCLE-TARGETING</i>							
ACE-031	ActRII inhibitor	N	N/A				DMD - NCT01099761 (Phase II): Trend towards improved muscular function and increased lean body mass. Study discontinued due to side-effects (telangiectasia and epistaxis)
Bimagrumab	ActRII inhibitor	N	N/A				Sporadic IBM - NCT01925209 (Phase IIb): No functional improvement NCT02573467 (Phase III): Long-term extension of same study (2 years) did not show any functional benefit.

							Sarcopenia - NCT02333331 (Phase II): no significant functional benefit
Domagrozumab	Myostatin inhibitor	N	N/A				DMD - NCT02310763 and NCT02907619 (Phase II): no significant functional improvement
BIIB110	ActRIIA/B ligand trap	N	-	I	-	No results posted ¹⁵¹	
Apitegromab (SRK-015)	Selective myostatin inhibitor	N	NCT03921528	II	Type 2/3	Prelim. Results indicate improved HFMSE score	
Tirasemtiv (CK-2017357)	FSTAs	N	N/A				ALS - NCT02496767 (Phase III): no significant difference in the primary outcome measure (SVC) or any secondary outcome measures. Poor tolerability.
Reldesemtiv (CK-2127107)	FSTAs	N	NCT02644668	II	Types 2/3/4	Improved maximum expiratory pressure in the highest dose group. Post-hoc analysis also showed a significant positive change in the 6MWD at 4 weeks, but this was not significant at 8 weeks (p=0.058). ¹⁵²	N/A
Somatotropin (somatropin; GH)	Anabolic effect	Y (GH deficiency)	NCT00533221	II	Type 2/3	No significant effect on muscle strength and function	N/A
Recombinant IGF-1 (Mecasermin)	Anabolic effect	Y (growth failure)	N/A				ALS - Cochrane systematic review showed a slight but significant difference in AALSRS total score (based on 2 clinical trials). The third study included in the meta-analysis did not show any significant difference in muscle strength. The quality of all three clinical trials was low ¹⁵³ DMD - NCT01207908 (Phase II): increase in lean mass but no significant difference in muscle function
BVS857	IGF-1 mimetic	N	N/A				SBMA - NCT02024932 (Phase II): significant difference in thigh muscle volume, but no difference in muscle strength and function

Leuprorelin	Gonadotropin releasing hormone (GnRH) analogue	N	N/A				SBMA - UMIN000000474 (Phase II): significant delay in functional decline and a decrease in the incidence of pneumonia and death
<i>NEUROMUSCULAR JUNCTION</i>							
Pyridostigmine	AChE inhibitor	Y (MG)	NCT02941328	II	Types 2/3/4	Trial completed but final results not yet published. Preliminary reports show a reduction in fatigability ⁹⁴	N/A
Salbutamol	β 2-adrenoreceptor agonists	Y (asthma)	No large-scale clinical trials. Small clinical studies or case reports in SMA type 2 and 3 suggest a benefit on motor ⁹⁸ and respiratory function. ¹⁵⁴				MG - NCT03914638 (Phase II/III): recruiting FSHD - NCT00027391: results not posted. Previous trial did not show any improvement in muscle function ¹⁵⁵
4-aminopyridine (4-AP)	Blocking K ⁺ channels	Y (MS)	NCT01645787	II/III	Type 3	No improvement on motor function (6MWT distance, fatigue)	PLS - NCT02868567 (Phase I): active
Amifampridine (3,4-DAP)	Blocking K ⁺ channels	Y (Lambert-Eaton myasthenic syndrome)	NCT03781479	II	Type 3	No results posted	MG - NCT03579966 (Phase III): active
<i>CYTOSKELETON</i>							
Fasudil	ROCK-inhibition	Yes (limited countries only – prevention and treatment of cerebral vasospasm)	N/A				ALS - NCT03792490 (phase II): currently recruiting
<i>CELL DEATH PATHWAYS</i>							
MW150	p38 α MAPK inhibitor	N	N/A				AD – (Phase I): ongoing
Celecoxib	NSAID (pain and inflammation)	Y	NCT02876094	II	Type 2/3	Study terminated; no results posted	ALS - NCT04165850 (Phase II)/ NCT00355576 (Phase II): results not posted

661 **Table 2 – Summary of drugs with repurposing potential in SMA.** These drugs are either licensed or currently in clinical trials for other
662 indications but also have a therapeutic effect in preclinical SMA models. With known safety profiles, these therapies could be “repurposed” for
663 SMA and so have a potentially faster route to the clinic. Information is accurate as of March 2021. 6MWT = 6 minute walk test, AChE =
664 acetylcholinesterase, ActRII = activin type 2 receptors, AD = Alzheimer’s disease, ALS = amyotrophic lateral sclerosis, DMD = Duchenne

665 muscular dystrophy, FSTA = fast skeletal muscle troponin activator, GH = growth hormone, HDAC = histone deacetylase, IBM = inclusion
666 body myositis, MG = myasthenia gravis, MS = multiple sclerosis, PLS = primary lateral sclerosis, PGK1 = phosphoglycerate kinase 1, SBMA =
667 spinal-bulbar muscular atrophy, VGCC = voltage gated calcium channel
668

- 669 1. Verhaart IEC, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, Cook
670 SF, Lochmüller H. Prevalence, incidence and carrier frequency of 5q-linked spinal
671 muscular atrophy - A literature review. *Orphanet J Rare Dis.* 2017;12(1):1–15.
- 672 2. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, et al.
673 Identification and characterization of a spinal muscular atrophy-determining gene.
674 *Cell.* 1995;80(1):155–65.
- 675 3. Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AHM,
676 McPherson JD. A single nucleotide difference that alters splicing patterns
677 distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet.*
678 1999;8(7):1177–83.
- 679 4. Lorson CL, Androphy EJ. An exonic enhancer is required for inclusion of an essential
680 exon in the SMA-determining gene SMN. *Hum Mol Genet.* 2000;9(2):259–66.
- 681 5. Chaytow H, Huang YT, Gillingwater TH, Faller KMEE. The role of survival motor
682 neuron protein (SMN) in protein homeostasis. *Cell Mol Life Sci.*
683 2018;75(0123456789):1–18.
- 684 6. Hamilton G, Gillingwater TH. Spinal muscular atrophy: Going beyond the motor
685 neuron. *Trends Mol Med.* 2013;19(1):40–50.
- 686 7. Wadman RI, Jansen MD, Stam M, Wijngaarde CA, Curial CAD, Medic J, et al.
687 Intragenic and structural variation in the SMN locus and clinical variability in spinal
688 muscular atrophy. *Brain Commun.* 2020;2(2):1–13.
- 689 8. Singh NK, Singh NN, Androphy EJ, Singh RN. Splicing of a Critical Exon of Human
690 Survival Motor Neuron Is Regulated by a Unique Silencer Element Located in the Last
691 Intron. *Mol Cell Biol.* 2006;26(4):1333–46.
- 692 9. Hua Y, Vickers TA, Okunola HL, Bennett CF, Krainer AR. Antisense Masking of an
693 hnRNP A1/A2 Intronic Splicing Silencer Corrects SMN2 Splicing in Transgenic Mice.

- 694 Am J Hum Genet. 2008 Apr;82(4):834–48.
- 695 10. Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, Bennett CF, Krainer AR. Antisense
696 correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse
697 model. Genes Dev. 2010 Aug 1;24(15):1634–44.
- 698 11. Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett CF, Krainer AR. Peripheral
699 SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy
700 mouse model. Nature. 2011 Oct 5;478(7367):123–6.
- 701 12. Passini MA, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, et al. Antisense
702 Oligonucleotides Delivered to the Mouse CNS Ameliorate Symptoms of Severe Spinal
703 Muscular Atrophy. Sci Transl Med. 2011;3(72).
- 704 13. Rigo F, Chun SJ, Norris DA, Hung G, Lee S, Matson J, et al. Pharmacology of a
705 Central Nervous System Delivered 2'-O -Methoxyethyl-Modified Survival of Motor
706 Neuron Splicing Oligonucleotide in Mice and Nonhuman Primates. J Pharmacol Exp
707 Ther. 2014 Jul;350(1):46–55.
- 708 14. Osman EY, Miller MR, Robbins KL, Lombardi AM, Atkinson AK, Brehm AJ, Lorson
709 CL. Morpholino antisense oligonucleotides targeting intronic repressor Element1
710 improve phenotype in SMA mouse models. Hum Mol Genet. 2014 Sep
711 15;23(18):4832–45.
- 712 15. Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, et al. Treatment
713 of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-
714 escalation study. Lancet. 2016;388(10063):3017–26.
- 715 16. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, et al.
716 Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J
717 Med. 2017;377(18):1723–32.
- 718 17. Darras BT, Chiriboga CA, Iannaccone ST, Swoboda KJ, Montes J, Mignon L, et al.

- 719 Nusinersen in later-onset spinal muscular atrophy: Long-term results from the phase
720 1/2 studies. *Neurology*. 2019;92(21):e2492–506.
- 721 18. De Vivo DC, Bertini E, Swoboda KJ, Hwu WL, Crawford TO, Finkel RS, et al.
722 Nusinersen initiated in infants during the presymptomatic stage of spinal muscular
723 atrophy: Interim efficacy and safety results from the Phase 2 NURTURE study.
724 *Neuromuscul Disord*. 2019;29(11):842–56.
- 725 19. Audic F, De La Banda MGG, Bernoux D, Ramirez-Garcia P, Durigneux J, Barnerias
726 C, et al. Effects of nusinersen after one year of treatment in 123 children with SMA
727 type 1 or 2: A French real-life observational study. *Orphanet J Rare Dis*. 2020;15(1):1–
728 10.
- 729 20. Lavie M, Diamant N, Cahal M, Sadot E, Be'er M, Fattal-Valevski A, Sagi L, Domany
730 KA, Amirav I. Nusinersen for spinal muscular atrophy type 1: Real-world respiratory
731 experience. *Pediatr Pulmonol*. 2021;56(1):291–8.
- 732 21. Maggi L, Bello L, Bonanno S, Govoni A, Caponnetto C, Passamano L, et al.
733 Nusinersen safety and effects on motor function in adult spinal muscular atrophy type
734 2 and 3. *J Neurol Neurosurg Psychiatry*. 2020;91(11):1166–74.
- 735 22. Pane M, Coratti G, Sansone VA, Messina S, Catteruccia M, Bruno C, et al. Type I
736 SMA “new natural history”: long-term data in nusinersen-treated patients. *Ann Clin
737 Transl Neurol*. 2021;548–57.
- 738 23. Ramos DM, d’Ydewalle C, Gabbeta V, Dakka A, Klein SK, Norris DA, et al. Age-
739 dependent SMN expression in disease-relevant tissue and implications for SMA
740 treatment. *J Clin Invest*. 2019;129(11):4817–31.
- 741 24. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK.
742 Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat
743 Biotechnol*. 2009 Jan 21;27(1):59–65.

- 744 25. Meyer K, Ferraiuolo L, Schmelzer L, Braun L, McGovern V, Likhite S, et al.
745 Improving Single Injection CSF Delivery of AAV9-mediated Gene Therapy for SMA:
746 A Dose–response Study in Mice and Nonhuman Primates. *Mol Ther.* 2015
747 Mar;23(3):477–87.
- 748 26. Valori CF, Ning K, Wyles M, Mead RJ, Grierson AJ, Shaw PJ, Azzouz M. Systemic
749 Delivery of scAAV9 Expressing SMN Prolongs Survival in a Model of Spinal
750 Muscular Atrophy. *Sci Transl Med.* 2010 Jun 9;2(35):35ra42-35ra42.
- 751 27. Foust KD, Wang X, MCGovern VL, Braun L, Bevan AK, Haidet AM, et al. Rescue of
752 the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of
753 SMN. *Nat Biotechnol.* 2010;28(3):271–4.
- 754 28. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al.
755 Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N Engl J Med.*
756 2017;377(18):1713–22.
- 757 29. Hinderer C, Katz N, Buza EL, Dyer C, Goode T, Bell P, Richman LK, Wilson JM.
758 Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous
759 Administration of an Adeno-Associated Virus Vector Expressing Human SMN. *Hum*
760 *Gene Ther.* 2018;29(3):285–98.
- 761 30. Day JW, Finkel RS, Chiriboga CA, Connolly AM, Crawford TO, Darras BT, et al.
762 Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal
763 muscular atrophy in patients with two copies of SMN2 (STRIVE): an open-label,
764 single-arm, multicentre, phase 3 trial. *Lancet Neurol.* 2021;20(4):284–93.
- 765 31. Chand D, Mohr F, McMillan H, Tukov FF, Montgomery K, Kleyn A, Sun R,
766 Tauscher-Wisniewski S, Kaufmann P, Kullak-Ublick G. Hepatotoxicity following
767 administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal
768 muscular atrophy. *J Hepatol.* 2021;74(3):560–6.

- 769 32. Sivaramakrishnan M, McCarthy KD, Campagne S, Huber S, Meier S, Augustin A, et
770 al. Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule
771 splicing modifiers. *Nat Commun.* 2017;8(1):1476.
- 772 33. Kletzl H, Marquet A, Günther A, Tang W, Heuberger J, Groeneveld GJ, et al. The oral
773 splicing modifier RG7800 increases full length survival of motor neuron 2 mRNA and
774 survival of motor neuron protein: Results from trials in healthy adults and patients with
775 spinal muscular atrophy. *Neuromuscul Disord.* 2019;29(1):21–9.
- 776 34. Ratni H, Ebeling M, Baird J, Bendels S, Bylund J, Chen KS, et al. Discovery of
777 Risdiplam, a Selective Survival of Motor Neuron-2 (SMN2) Gene Splicing Modifier
778 for the Treatment of Spinal Muscular Atrophy (SMA). *J Med Chem.* 2018 Aug
779 9;61(15):6501–17.
- 780 35. Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, et al. SMN2
781 splicing modifiers improve motor function and longevity in mice with spinal muscular
782 atrophy. *Science (80-).* 2014 Aug 8;345(6197):688–93.
- 783 36. Poirier A, Weetall M, Heinig K, Bucheli F, Schoenlein K, Alsenz J, et al. Risdiplam
784 distributes and increases <sc>SMN</sc> protein in both the central nervous system
785 and peripheral organs. *Pharmacol Res Perspect.* 2018 Dec 29;6(6):e00447.
- 786 37. Sturm S, Günther A, Jaber B, Jordan P, Al Kotbi N, Parkar N, et al. A phase 1 healthy
787 male volunteer single escalating dose study of the pharmacokinetics and
788 pharmacodynamics of risdiplam (RG7916, RO7034067), a SMN2 splicing modifier.
789 *Br J Clin Pharmacol.* 2019;85(1):181–93.
- 790 38. Baranello G, Darras BT, Day JW, Deconinck N, Klein A, Masson R, et al. Risdiplam
791 in Type 1 Spinal Muscular Atrophy. *N Engl J Med.* 2021 Feb 24;NEJMoa2009965.
- 792 39. Sergott RC, Amorelli GM, Baranello G, Barreau E, Beres S, Kane S, et al. Risdiplam
793 treatment has not led to retinal toxicity in patients with spinal muscular atrophy. *Ann*

- 794 Clin Transl Neurol. 2021;8(1):54–65.
- 795 40. Palacino J, Swalley SE, Song C, Cheung AK, Shu L, Zhang X, et al. SMN2 splice
796 modulators enhance U1–pre-mRNA association and rescue SMA mice. Nat Chem
797 Biol. 2015 Jul 1;11(7):511–7.
- 798 41. Novartis receives US Food and Drug Administration (FDA) Orphan Drug Designation
799 for branaplam (LMI070) in Huntington’s disease (HD) [Internet]. Novartis.
- 800 42. Ando S, Suzuki S, Okubo S, Ohuchi K, Takahashi K, Nakamura S, Shimazawa M,
801 Fuji K, Hara H. Discovery of a CNS penetrant small molecule SMN2 splicing
802 modulator with improved tolerability for spinal muscular atrophy. Sci Rep.
803 2020;10(1):1–16.
- 804 43. Younis I, Berg M, Kaida D, Dittmar K, Wang C, Dreyfuss G. Rapid-Response
805 Splicing Reporter Screens Identify Differential Regulators of Constitutive and
806 Alternative Splicing. Mol Cell Biol. 2010;30(7):1718–28.
- 807 44. Sapaly D, Dos Santos M, Delers P, Biondi O, Quérol G, Houdebine L, et al. Small-
808 molecule flunarizine increases SMN protein in nuclear Cajal bodies and motor
809 function in a mouse model of spinal muscular atrophy. Sci Rep. 2018 Dec 1;8(1):2075.
- 810 45. Osman EY, Washington CW, Kaifer KA, Mazzasette C, Patitucci TN, Florea KM,
811 Simon ME, Ko C-P, Ebert AD, Lorson CL. Optimization of Morpholino Antisense
812 Oligonucleotides Targeting the Intronic Repressor Element1 in Spinal Muscular
813 Atrophy. Mol Ther. 2016 Sep;24(9):1592–601.
- 814 46. Hammond SM, Hazell G, Shabanpoor F, Saleh AF, Bowerman M, Sleigh JN, et al.
815 Systemic peptide-mediated oligonucleotide therapy improves long-term survival in
816 spinal muscular atrophy. Proc Natl Acad Sci. 2016;113(39):10962–7.
- 817 47. Matesanz SE, Battista V, Flickinger J, Jones JN, Kichula EA. Clinical Experience with
818 Gene Therapy in Older Spinal Muscular Atrophy Patients. Pediatr Neurol.

- 819 2021;118(July 2019):1–5.
- 820 48. Darrow JJ, Sharma M, Shroff M, Wagner AK. Efficacy and costs of spinal muscular
821 atrophy drugs. *Sci Transl Med.* 2020;12(569):9–12.
- 822 49. Brichta L, Hofmann Y, Hahnen E, Siebzehnrubi FA, Raschke H, Blumcke I, Eyupoglu
823 IY, Wirth B. Valproic acid increases the SMN2 protein level: A well-known drug as a
824 potential therapy for spinal muscular atrophy. *Hum Mol Genet.* 2003;12(19):2481–9.
- 825 50. Sumner CJ, Huynh TN, Markowitz JA, Perhac JS, Hill B, Coover DD, et al. Valproic
826 Acid Increases SMN Levels in Spinal Muscular Atrophy Patient Cells. *Ann Neurol.*
827 2003;54(5):647–54.
- 828 51. Andreassi C, Angelozzi C, Tiziano FD, Vitali T, De Vincenzi E, Boninsegna A, et al.
829 Phenylbutyrate increases SMN expression in vitro: Relevance for treatment of spinal
830 muscular atrophy. *Eur J Hum Genet.* 2004;12(1):59–65.
- 831 52. Lai JI, Leman LJ, Ku S, Vickers CJ, Olsen CA, Montero A, Ghadiri MR, Gottesfeld
832 JM. Cyclic tetrapeptide HDAC inhibitors as potential therapeutics for spinal muscular
833 atrophy: Screening with iPSC-derived neuronal cells. *Bioorganic Med Chem Lett.*
834 2017;27(15):3289–93.
- 835 53. Tsai LK, Tsai MS, Ting CH, Li H. Multiple therapeutic effects of valproic acid in
836 spinal muscular atrophy model mice. *J Mol Med.* 2008;86(11):1243–54.
- 837 54. Elshafay A, Hieu TH, Doheim MF, Kassem MAM, ELdoadoa MF, Holloway SK,
838 Abo-elghar H, Hirayama K, Huy NT. Efficacy and Safety of Valproic Acid for Spinal
839 Muscular Atrophy: A Systematic Review and Meta-Analysis. *CNS Drugs.*
840 2019;33(3):239–50.
- 841 55. Brahe C, Vitali T, Tiziano FD, Angelozzi C, Pinto AM, Borgo F, Moscato U, Bertini
842 E, Mercuri E, Neri G. Phenylbutyrate increases SMN gene expression in spinal
843 muscular atrophy patients. *Eur J Hum Genet.* 2005;13(2):256–9.

- 844 56. Hauke J, Riessland M, Lunke S, Eyüpoglu IY, Blümcke I, El-osta A, Wirth B, Hahnen
845 E. Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal
846 muscular atrophy disease severity and can be bypassed by histone deacetylase
847 inhibition. *Hum Mol Genet.* 2009;18(2):304–17.
- 848 57. Narver HL, Kong L, Burnett BG, Choe DW, Bosch-Marcé M, Taye AA, Eckhaus MA,
849 Sumner CJ. Sustained improvement of spinal muscular atrophy mice treated with
850 trichostatin A plus nutrition. *Ann Neurol.* 2008;64(4):465–70.
- 851 58. Dayangaç-Erden D, Bora G, Ayhan P, Kocaeffe Ç, Dalkara S, Yelekçi K, Demir AS,
852 Erdem-Yurter H. Histone deacetylase inhibition activity and molecular docking of (e)-
853 resveratrol: Its therapeutic potential in spinal muscular atrophy. *Chem Biol Drug Des.*
854 2009;73(3):355–64.
- 855 59. Groen EJM, Talbot K, Gillingwater TH. Advances in therapy for spinal muscular
856 atrophy: promises and challenges. *Nat Rev Neurol.* 2018;
- 857 60. Pagliarini V, Guerra M, Di Rosa V, Compagnucci C, Sette C. Combined treatment
858 with the histone deacetylase inhibitor LBH589 and a splice-switch antisense
859 oligonucleotide enhances SMN2 splicing and SMN expression in Spinal Muscular
860 Atrophy cells. *J Neurochem.* 2020;153(2):264–75.
- 861 61. Jangi M, Fleet C, Cullen P, Gupta S V., Mekhoubad S, Chiao E, et al. SMN deficiency
862 in severe models of spinal muscular atrophy causes widespread intron retention and
863 DNA damage. *Proc Natl Acad Sci U S A.* 2017;114(12):E2347–56.
- 864 62. Kannan A, Bhatia K, Branzei D, Gangwani L. Combined deficiency of Senataxin and
865 DNA-PKcs causes DNA damage accumulation and neurodegeneration in spinal
866 muscular atrophy. *Nucleic Acids Res.* 2018;46(16):8326–46.
- 867 63. Kannan A, Jiang X, He L, Ahmad S, Gangwani L. ZPR1 prevents R-loop
868 accumulation, upregulates SMN2 expression and rescues spinal muscular atrophy.

- 869 Brain. 2020;143(1):69–93.
- 870 64. Lunn MR, Root DE, Martino AM, Flaherty SP, Kelley BP, Coovert DD, et al.
871 Indoprofen upregulates the survival motor neuron protein through a cyclooxygenase-
872 independent mechanism. *Chem Biol.* 2004;11(11):1489–93.
- 873 65. Osman EY, Rietz A, Kline RA, Cherry JJ, Hodgetts KJ, Lorson CL, Androphy EJ.
874 Intraperitoneal delivery of a novel drug-like compound improves disease severity in
875 severe and intermediate mouse models of Spinal Muscular Atrophy. *Sci Rep.* 2019
876 Dec 7;9(1):1633.
- 877 66. Mattis VB, Rai R, Wang J, Chang CWT, Coady T, Lorson CL. Novel aminoglycosides
878 increase SMN levels in spinal muscular atrophy fibroblasts. *Hum Genet.*
879 2006;120(4):589–601.
- 880 67. Mattis VB, Tom Chang C-W, Lorson CL. Analysis of a read-through promoting
881 compound in a severe mouse model of spinal muscular atrophy. *Neurosci Lett.* 2012
882 Sep;525(1):72–5.
- 883 68. Osman EY, Washington CW, Simon ME, Megidido D, Greif H, Lorson CL. Analysis
884 of Azithromycin Monohydrate as a Single or a Combinatorial Therapy in a Mouse
885 Model of Severe Spinal Muscular Atrophy. *J Neuromuscul Dis.* 2017 Aug
886 29;4(3):237–49.
- 887 69. Foran E, Kwon DY, Nofziger JH, Arnold ES, Hall MD, Fischbeck KH, Burnett BG.
888 CNS uptake of bortezomib is enhanced by P-glycoprotein inhibition: implications for
889 spinal muscular atrophy. *Neurobiol Dis.* 2016 Apr;88(1):118–24.
- 890 70. Boyd PJ, Tu W, Shorrock HK, Groen EJN, Carter N, Powis RA, et al. Bioenergetic
891 status modulates motor neuron vulnerability and pathogenesis in a zebrafish model of
892 spinal muscular atrophy. *PLOS Genet.* 2017;13(4):1–27.
- 893 71. Bordet T, Berna P, Abitbol JL, Pruss RM. Olesoxime (TRO19622): A novel

- 894 mitochondrial-targeted neuroprotective compound. *Pharmaceuticals*. 2010;3(2):345–
895 68.
- 896 72. Bertini E, Dessaud E, Mercuri E, Muntoni F, Kirschner J, Reid C, et al. Safety and
897 efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular
898 atrophy: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*.
899 2017;16(7):513–22.
- 900 73. Weber JJ, Clemensson LE, Schiöth HB, Nguyen HP. Olesoxime in neurodegenerative
901 diseases: Scrutinising a promising drug candidate. *Biochem Pharmacol*.
902 2019;168(May):305–18.
- 903 74. Russman BS, Iannaccone ST, Samaha FJ. A Phase 1 Trial of Riluzole in Spinal
904 Muscular Atrophy. *Arch Neurol*. 2003;60(11):1601–3.
- 905 75. Merlini L, Solari A, Vita G, Bertini E, Minetti C, Mongini T, Mazzoni E, Angelini C,
906 Morandi L. Role of gabapentin in spinal muscular atrophy: Results of a multicenter,
907 randomized Italian study. *J Child Neurol*. 2003;18(8):537–41.
- 908 76. Miller RG, Moore DH, Dronsky V, Bradley W, Barohn R, Bryan W, et al. A placebo-
909 controlled trial of gabapentin in spinal muscular atrophy. *J Neurol Sci*. 2001;191(1–
910 2):127–31.
- 911 77. Ando S, Funato M, Ohuchi K, Kameyama T, Inagaki S, Seki J, et al. Edaravone is a
912 candidate agent for spinal muscular atrophy: In vitro analysis using a human induced
913 pluripotent stem cells-derived disease model. *Eur J Pharmacol*. 2017;814(April):161–
914 8.
- 915 78. Ando S, Funato M, Ohuchi K, Inagaki S, Sato A, Seki J, et al. The Protective Effects
916 of Levetiracetam on a Human iPSCs-Derived Spinal Muscular Atrophy Model.
917 *Neurochem Res*. 2019;44(7):1773–9.
- 918 79. Kim J-K, Jha NN, Feng Z, Faleiro MR, Chiriboga CA, Wei-Lapierre L, Dirksen RT,

- 919 Ko C-P, Monani UR. Muscle-specific SMN reduction reveals motor neuron–
920 independent disease in spinal muscular atrophy models. *J Clin Invest*.
921 2020;130(3):1271–87.
- 922 80. Zhou H, Meng J, Malerba A, Catapano F, Sintusek P, Jarmin S, et al. Myostatin
923 inhibition in combination with antisense oligonucleotide therapy improves outcomes in
924 spinal muscular atrophy. *J Cachexia Sarcopenia Muscle*. 2020;11(3):768–82.
- 925 81. Liu M, Hammers DW, Barton ER, Sweeney HL. Activin receptor type IIB inhibition
926 improves muscle phenotype and function in a mouse model of spinal muscular
927 atrophy. *PLoS One*. 2016;11(11):1–17.
- 928 82. Long KK, O’Shea KM, Khairallah RJ, Howell K, Paushkin S, Chen KS, et al. Specific
929 inhibition of myostatin activation is beneficial in mouse models of SMA therapy. *Hum*
930 *Mol Genet*. 2019;28(7):1076–89.
- 931 83. Rose FF, Mattis VB, Rindt H, Lorson CL. Delivery of recombinant follistatin lessens
932 disease severity in a mouse model of spinal muscular atrophy. *Hum Mol Genet*.
933 2009;18(6):997–1005.
- 934 84. Campbell C, McMillan HJ, Mah JK, Tarnopolsky M, Selby K, McClure T, Wilson
935 DM, Sherman ML, Escolar D, Attie KM. Myostatin inhibitor ACE-031 treatment of
936 ambulatory boys with Duchenne muscular dystrophy: Results of a randomized,
937 placebo-controlled clinical trial. *Muscle and Nerve*. 2017;55(4):458–64.
- 938 85. Scholar Rock Announces Positive Proof-of-Concept Data from TOPAZ Phase 2 Trial
939 Interim Analysis of SRK-015 in Patients with Type 2 and Type 3 Spinal Muscular
940 Atrophy [Internet]. Scholar Rock.
- 941 86. Cheng AJ, Hwee DT, Kim LH, Durham N, Yang HT, Hinken AC, et al. Fast skeletal
942 muscle troponin activator CK-2066260 increases fatigue resistance by reducing the
943 energetic cost of muscle contraction. *J Physiol*. 2019;597(17):4615–25.

- 944 87. Tsai LK, Chen CL, Ting CH, Lin-Chao S, Hwu WL, Dodge JC, Passini MA, Cheng
945 SH. Systemic administration of a recombinant AAV1 vector encoding IGF-1 improves
946 disease manifestations in SMA mice. *Mol Ther.* 2014;22(8):1450–9.
- 947 88. Kirschner J, Schorling D, Hauschke D, Rensing-Zimmermann C, Wein U, Grieben U,
948 et al. Somatropin treatment of spinal muscular atrophy: A placebo-controlled, double-
949 blind crossover pilot study. *Neuromuscul Disord.* 2014;24(2):134–42.
- 950 89. Ahlskog N, Hayler D, Krueger A, Kubinski S, Claus P, Hammond SM, Wood MJA,
951 Yáñez-Muñoz RJ, Bowerman M. Muscle overexpression of Klf15 via an AAV8-Spc5-
952 12 construct does not provide benefits in spinal muscular atrophy mice. *Gene Ther.*
953 2020;505–15.
- 954 90. Kim JK, Caine C, Awano T, Herbst R, Monani UR. Motor neuronal repletion of the
955 NMJ organizer, Agrin, modulates the severity of the spinal muscular atrophy disease
956 phenotype in model mice. *Hum Mol Genet.* 2017;26(13):2377–85.
- 957 91. Kaifer KA, Villalón E, Smith CE, Simon ME, Marquez J, Hopkins AE, Morcos TI,
958 Lorson CL. AAV9-DOK7 gene therapy reduces disease severity in *Smn2B^{-/-}* SMA
959 model mice. *Biochem Biophys Res Commun.* 2020;530(1):107–14.
- 960 92. Boido M, de Amicis E, Valsecchi V, Trevisan M, Ala U, Ruegg MA, Hettwer S,
961 Vercelli A. Increasing agrin function antagonizes muscle atrophy and motor
962 impairment in spinal muscular atrophy. *Front Cell Neurosci.* 2018;12(January):1–14.
- 963 93. Wadman RI, Vrancken AFJE, van den Berg LH, van der Pol WL. Dysfunction of the
964 neuromuscular junction in spinal muscular atrophy types 2 and 3. *Neurology.* 2012
965 Nov 13;79(20):2050–5.
- 966 94. Stam M, Wadman RI, Wijngaarde CA, Bartels B, Asselman FL, Otto LAM, et al.
967 Protocol for a phase II, monocentre, double-blind, placebo-controlled, cross-over trial
968 to assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2-4

- 969 (SPACE trial). *BMJ Open*. 2018;8(7):1–9.
- 970 95. Stam M, Wijngaarde CA, Bartels B, Asselman F-L, Otto LAM, Goedee S, et al. Space
971 trial. A phase II, monocenter, double-blind, placebo-controlled, cross-over trial to
972 assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2, 3
973 and 4. In: *Proceedings of the 23rd Cure SMA researcher meeting*. 2019.
- 974 96. Tiziano FD, Lomastro R, Pinto AM, Messina S, Amico AD, Fiori S, et al. Salbutamol
975 increases survival motor neuron (SMN) transcript levels in leucocytes of spinal
976 muscular atrophy (SMA) patients : relevance for clinical trial design. *J Med Genet*.
977 2010;47:856–9.
- 978 97. Clausen L, Cossins J, Beeson D. Beta-2 Adrenergic Receptor Agonists Enhance AChR
979 Clustering in C2C12 Myotubes: Implications for Therapy of Myasthenic Disorders. *J*
980 *Neuromuscul Dis*. 2018;5(2):231–40.
- 981 98. Frongia AL, Natera-de Benito D, Ortez C, Alarcón M, Borrás A, Medina J, et al.
982 Salbutamol tolerability and efficacy in patients with spinal muscular atrophy type II.
983 *Neuromuscul Disord*. 2019;29(7):517–24.
- 984 99. Khirani S, Dabaj I, Amaddeo A, Olmo Arroyo J, Ropers J, Tirolien S, Coudert V,
985 Estournet B, Fauroux B, Quijano-Roy S. Effect of Salbutamol on Respiratory Muscle
986 Strength in Spinal Muscular Atrophy. *Pediatr Neurol*. 2017;73:78-87.e1.
- 987 100. Imlach WL, Beck ES, Choi BJ, Lotti F, Pellizzoni L, McCabe BD. SMN is required
988 for sensory-motor circuit function in drosophila. *Cell*. 2012;151(2):427–39.
- 989 101. Chiriboga CA, Marra J, LaMarca NM, Young SD, Weimer LH, Levin B, McCabe B.
990 Lack of effect on ambulation of dalfampridine-ER (4-AP) treatment in adult SMA
991 patients. *Neuromuscul Disord*. 2020;30(8):693–700.
- 992 102. Tejero R, Lopez-Manzaneda M, Arumugam S, Tabares L. Synaptotagmin-2, and -1,
993 linked to neurotransmission impairment and vulnerability in Spinal Muscular Atrophy.

- 994 Hum Mol Genet. 2016;25(21):4703–16.
- 995 103. Tejero R, Balk S, Franco-Espin J, Ojeda J, Hennlein L, Drexler H, et al. R-Roscovitin
996 Improves Motoneuron Function in Mouse Models for Spinal Muscular Atrophy.
997 iScience. 2020;23(2):100826.
- 998 104. Nolle A, Zeug A, Van bergeijk J, Tonges L, Gerhard R, Brinkmann H, et al. The spinal
999 muscular atrophy disease protein SMN is linked to the Rho-kinase pathway via
1000 profilin. Hum Mol Genet. 2011;20(24):4865–78.
- 1001 105. Bowerman M, Beauvais A, Anderson CL, Kothary R. Rho-kinase inactivation
1002 prolongs survival of an intermediate SMA mouse model. Hum Mol Genet.
1003 2010;19(8):1468–78.
- 1004 106. Bowerman M, Murray LM, Boyer JG, Anderson CL, Kothary R. Fasudil improves
1005 survival and promotes skeletal muscle development in a mouse model of spinal
1006 muscular atrophy. BMC Med. 2012;10:1–14.
- 1007 107. Little D, Valori CF, Mutsaers CA, Bennett EJ, Wyles M, Sharrack B, Shaw PJ,
1008 Gillingwater TH, Azzouz M, Ning K. PTEN depletion decreases disease severity and
1009 modestly prolongs survival in a mouse model of spinal muscular atrophy. Mol Ther.
1010 2015;23(2):270–7.
- 1011 108. Wen H-L, Lin Y-T, Ting C-H, Lin-Chao S, Li H, Hsieh-Li HM. Stathmin, a
1012 microtubule-destabilizing protein, is dysregulated in spinal muscular atrophy. Hum
1013 Mol Genet. 2010 May 1;19(9):1766–78.
- 1014 109. Hosseinibarkooie S, Peters M, Torres-Benito L, Rastetter RHH, Hupperich K,
1015 Hoffmann A, et al. The Power of Human Protective Modifiers: PLS3 and CORO1C
1016 Unravel Impaired Endocytosis in Spinal Muscular Atrophy and Rescue SMA
1017 Phenotype. Am J Hum Genet. 2016;99(3):647–65.
- 1018 110. Riessland M, Kaczmarek A, Schneider S, Swoboda KJ, Löhr H, Bradler C, et al.

- 1019 Neurocalcin Delta Suppression Protects against Spinal Muscular Atrophy in Humans
1020 and across Species by Restoring Impaired Endocytosis. *Am J Hum Genet.*
1021 2017;100(2):297–315.
- 1022 111. Oprea GE, Kröber S, McWhorter ML, Rossoll W, Müller S, Krawczak M, Bassell GJ,
1023 Beattie CE, Wirth B. Plastin 3 is a protective modifier of autosomal recessive spinal
1024 muscular atrophy. *Science* (80-). 2008;320(5875):524–7.
- 1025 112. McGovern VL, Massoni-Laporte A, Wang X, Le TT, Le HT, Beattie CE, Rich MM,
1026 Burghes AHM. Plastin 3 expression does not modify spinal muscular atrophy severity
1027 in the $\Delta 7$ SMA mouse. *PLoS One.* 2015;10(7):1–19.
- 1028 113. Torres-Benito L, Schneider S, Rombo R, Ling KK, Gysko V, Upadhyay A,
1029 Kononenko NL, Rigo F, Bennett CF, Wirth B. NCALD Antisense Oligonucleotide
1030 Therapy in Addition to Nusinersen further Ameliorates Spinal Muscular Atrophy in
1031 Mice. *Am J Hum Genet.* 2019;105(1):221–30.
- 1032 114. Janzen E, Mendoza-Ferreira N, Hosseinibarkooie S, Schneider S, Hupperich K,
1033 Tschanz T, et al. CHP1 reduction ameliorates spinal muscular atrophy pathology by
1034 restoring calcineurin activity and endocytosis. *Brain.* 2018;141(8):2343–61.
- 1035 115. Walsh MB, Janzen E, Wingrove E, Hosseinibarkooie S, Muela NR, Davidow L, et al.
1036 Genetic modifiers ameliorate endocytic and neuromuscular defects in a model of
1037 spinal muscular atrophy. *BMC Biol.* 2020;18(1):127.
- 1038 116. Custer SK, Androphy EJ. Autophagy dysregulation in cell culture and animals models
1039 of spinal muscular atrophy. *Mol Cell Neurosci.* 2014;61:133–40.
- 1040 117. Piras A, Schiaffino L, Boido M, Valsecchi V, Guglielmotto M, De Amicis E, et al.
1041 Inhibition of autophagy delays motoneuron degeneration and extends lifespan in a
1042 mouse model of spinal muscular atrophy. *Cell Death Dis.* 2017;8(12):3223.
- 1043 118. Periyakaruppiyah A, de la Fuente S, Arumugam S, Bahí N, Garcera A, Soler RM.

- 1044 Autophagy modulators regulate survival motor neuron protein stability in
1045 motoneurons. *Exp Neurol*. 2016;283:287–97.
- 1046 119. de la Fuente S, Sansa A, Periyakarupiah A, Garcera A, Soler RM. Calpain Inhibition
1047 Increases SMN Protein in Spinal Cord Motoneurons and Ameliorates the Spinal
1048 Muscular Atrophy Phenotype in Mice. *Mol Neurobiol*. 2019;56(6):4414–27.
- 1049 120. Ramser J, Ahearn ME, Lenski C, Yariz KO, Hellebrand H, Rhein M Von, et al. Rare
1050 Missense and Synonymous Variants in UBE1 Are Associated with X-Linked Infantile
1051 Spinal Muscular Atrophy. *Am J Hum Genet*. 2008;82(January):188–93.
- 1052 121. Powis RA, Karyka E, Boyd P, Côme J, Jones RA, Zheng Y, et al. Systemic restoration
1053 of UBA1 ameliorates disease in spinal muscular atrophy. *JCI Insight*. 2016;1(11):1–
1054 16.
- 1055 122. Wishart TM, Mutsaers CA, Riessland M, Reimer MM, Hunter G, Hannam ML, et al.
1056 Disrupted ubiquitin homeostasis and beta-catenin signalling in spinal muscular
1057 atrophy. *J Clin Invest*. 2014;124(4):1821–34.
- 1058 123. Abera MB, Xiao J, Nofziger J, Titus S, Southall N, Zheng W, et al. ML372 blocks
1059 SMN ubiquitination and improves spinal muscular atrophy pathology in mice. *JCI*
1060 *insight*. 2016;1(19):e88427.
- 1061 124. Musi CA, Agrò G, Santarella F, Iervasi E, Borsello T. JNK3 as Therapeutic Target and
1062 Biomarker in Neurodegenerative and Neurodevelopmental Brain Diseases. *Cells*.
1063 2020;9(10):1–22.
- 1064 125. Genabai NK, Ahmad S, Zhang Z, Jiang X, Gabaldon CA, Gangwani L. Genetic
1065 inhibition of JNK3 ameliorates spinal muscular atrophy. *Hum Mol Genet*.
1066 2015;24(24):6986–7004.
- 1067 126. Pilato CM, Park JH, Kong L, D’Ydewalle C, Valdivia D, Chen KS, Griswold-Prenner
1068 I, Sumner CJ. Motor neuron loss in SMA is not associated with somal stress-activated

- 1069 JNK/c-Jun signaling. *Hum Mol Genet.* 2019;28(19):3283–92.
- 1070 127. Schellino R, Boido M, Borsello T, Vercelli A. Pharmacological c-Jun NH2-Terminal
1071 Kinase (JNK) Pathway Inhibition Reduces Severity of Spinal Muscular Atrophy
1072 Disease in Mice. *Front Mol Neurosci.* 2018;11(September):1–15.
- 1073 128. Simon CM, Van Alstyne M, Lotti F, Bianchetti E, Tisdale S, Watterson DM, Mentis
1074 GZ, Pellizzoni L. Stasimon Contributes to the Loss of Sensory Synapses and Motor
1075 Neuron Death in a Mouse Model of Spinal Muscular Atrophy. *Cell Rep.*
1076 2019;29(12):3885-3901.e5.
- 1077 129. Simon CM, Dai Y, Alstyne M Van, Henderson CE, Pellizzoni L, Mentis GZ, et al.
1078 Converging Mechanisms of p53 Activation Drive Motor Neuron Degeneration in
1079 Spinal Muscular Atrophy. *Neuron.* 2017;94(4):767–80.
- 1080 Neuron Degeneration in Spinal Muscular Atrophy. 2017;3767–80.
- 1081 130. Osman EY, van Alstyne M, Yen PF, Lotti F, Feng Z, Ling KKY, Ko CP, Pellizzoni L,
1082 Lorson CL. Minor snRNA gene delivery improves the loss of proprioceptive synapses
1083 on SMA motor neurons. *JCI Insight.* 2020;5(12).
- 1084 131. Farooq F, Abadía-Molina F, MacKenzie D, Hadwen J, Shamim F, O’Reilly S, Holcik
1085 M, MacKenzie A. Celecoxib increases SMN and survival in a severe spinal muscular
1086 atrophy mouse model via p38 pathway activation. *Hum Mol Genet.* 2013 Sep
1087 1;22(17):3415–24.
- 1088 132. Sleight JN, Barreiro-Iglesias A, Oliver PL, Biba A, Becker T, Davies KE, Becker CG,
1089 Talbot K. Chondrolectin affects cell survival and neuronal outgrowth in in vitro and in
1090 vivo models of spinal muscular atrophy. *Hum Mol Genet.* 2014;23(4):855–69.
- 1091 133. Oprea AM, Smith HL, Arya S, Webster R, Zhong Z, Wehner D, Cardozo MJ,
1092 Becker T, Talbot K, Becker CG. Interaction of Axonal Chondrolectin with Collagen
1093 XIXa1 Is Necessary for Precise Neuromuscular Junction Formation. *Cell Rep.*

- 1094 2019;29(5):1082-1098.e10.
- 1095 134. Mushtaq G, Greig NH, Anwar F, Al-Abbasi FA, Zamzami MA, Al-Talhi HA, Kamal
1096 MA. Neuroprotective Mechanisms Mediated by CDK5 Inhibition. *Curr Pharm Des.*
1097 2016;22(5):527–34.
- 1098 135. Miller N, Feng Z, Edens BM, Yang B, Shi H, Sze CC, et al. Non-aggregating tau
1099 phosphorylation by cyclin-dependent kinase 5 contributes to motor neuron
1100 degeneration in spinal muscular atrophy. *J Neurosci.* 2015;35(15):6038–50.
- 1101 136. Valsecchi V, Boido M, De Amicis E, Piras A, Vercelli A. Expression of muscle-
1102 specific MiRNA 206 in the progression of disease in a murine SMA model. *PLoS One.*
1103 2015;10(6):1–17.
- 1104 137. Valsecchi V, Anzilotti S, Serani A, Laudati G, Brancaccio P, Guida N, Cuomo O,
1105 Pignataro G, Annunziato L. miR-206 Reduces the Severity of Motor Neuron
1106 Degeneration in the Facial Nuclei of the Brainstem in a Mouse Model of SMA. *Mol*
1107 *Ther.* 2020;28(4):1154–66.
- 1108 138. Kaifer KA, Villalón E, O’Brien BS, Sison SL, Smith CE, Simon ME, et al. AAV9-
1109 mediated delivery of miR-23a reduces disease severity in *Smn2B^{-/-}*-SMA model mice.
1110 *Hum Mol Genet.* 2019;28(19):3199–210.
- 1111 139. Deguise M, Chehade L, Kothary R. Metabolic Dysfunction in Spinal Muscular
1112 Atrophy. *Int J Mol Sci.* 2021 May 31;22(11):5913.
- 1113 140. Houdebine L, D’Amico D, Bastin J, Chali F, Deseille C, Rumeau V, et al. Low-
1114 Intensity Running and High-Intensity Swimming Exercises Differentially Improve
1115 Energy Metabolism in Mice With Mild Spinal Muscular Atrophy. *Front Physiol.*
1116 2019;10(October):1–21.
- 1117 141. Dangouloff T, Servais L. Clinical evidence supporting early treatment of patients with
1118 spinal muscular atrophy: Current perspectives. *Ther Clin Risk Manag.* 2019;15:1153–

- 1119 61.
- 1120 142. Motyl A, Faller K, Groen E, Kline R, Eaton S, Ledahawsky L, et al. Pre-natal
1121 manifestation of systemic developmental abnormalities in spinal muscular atrophy.
1122 *Hum Mol Genet.* 2020;00(00):1–10.
- 1123 143. Hagenacker T, Wurster CD, Günther R, Schreiber-Katz O, Osmanovic A, Petri S, et al.
1124 Nusinersen in adults with 5q spinal muscular atrophy: a non-interventional,
1125 multicentre, observational cohort study. *Lancet Neurol.* 2020;19(4):317–25.
- 1126 144. Walter MC, Wenninger S, Thiele S, Stauber J, Hiebeler M, Greckl E, et al. Safety and
1127 treatment effects of nusinersen in longstanding adult 5q-SMA type 3 – A prospective
1128 observational study. *J Neuromuscul Dis.* 2019;6(4):453–65.
- 1129 145. Sheridan C. First oral SMA drug. *Nat Biotechnol.* 2020 Oct 5;38(10):1111–1111.
- 1130 146. Roche. Media & Investor Release [Internet]. [https://ml-](https://ml-eu.globenewswire.com/Resource/Download/75a9fb47-7113-47da-aca0-361ca5d6678a)
1131 [eu.globenewswire.com/Resource/Download/75a9fb47-7113-47da-aca0-361ca5d6678a.](https://ml-eu.globenewswire.com/Resource/Download/75a9fb47-7113-47da-aca0-361ca5d6678a)
1132 2021. p. 1–5.
- 1133 147. Charnas L, Voltz E, Pfister C, Peters T, Hartmann A, Berghs-Clairmont C, et al. Safety
1134 and efficacy findings in the first-in-human trial (FIH) of the oral splice modulator
1135 branaplam in type 1 spinal muscular atrophy (SMA): interim results. *Neuromuscul*
1136 *Disord.* 2017;27(2017):S207–8.
- 1137 148. Paganoni S, Hendrix S, Dickson SP, Knowlton N, Macklin EA, Berry JD, et al. Long-
1138 term survival of participants in the CENTAUR trial of sodium phenylbutyrate-
1139 taurursodiol in amyotrophic lateral sclerosis. *Muscle and Nerve.* 2021;63(1):31–9.
- 1140 149. Kawamura K, Fukumura S, Nikaido K, Tachi N, Kozuka N, Seino T, et al. Resveratrol
1141 improves motor function in patients with muscular dystrophies: an open-label, single-
1142 arm, phase IIa study. *Sci Rep.* 2020;10(1):1–11.
- 1143 150. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis

- 1144 (ALS)/motor neuron disease (MND). Cochrane database Syst Rev. 2012 Mar
1145 14;(3):CD001447.
- 1146 151. Biogen. Pipeline [Internet]. https://www.biogen.com/en_us/pipeline.html.
- 1147 152. Rudnicki SA, Andrews JA, Duong T, Cockroft BM, Malik FI, Meng L, et al.
1148 Reldesemtiv in Patients with Spinal Muscular Atrophy: a Phase 2 Hypothesis-
1149 Generating Study. *Neurotherapeutics*. 2021 Feb 23;
- 1150 153. Mitchell JD, Wokke JHJ, Borasio GD. Recombinant human insulin-like growth factor
1151 I (rhIGF-I) for amyotrophic lateral sclerosis/motor neuron disease. In: Mitchell JD,
1152 editor. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley &
1153 Sons, Ltd; 2007.
- 1154 154. Pera MC, Luigetti M, Sivo S, Lapenta L, Granata G, Antonaci L, Coratti G, Forcina N,
1155 Pane M, Mercuri E. Does albuterol have an effect on neuromuscular junction
1156 dysfunction in spinal muscular atrophy? *Neuromuscul Disord*. 2018;28(10):863–4.
- 1157 155. Payan CA, Hogrel JY, Hammouda EH, Lacomblez L, Ollivier G, Doppler V, et al.
1158 Periodic Salbutamol in Facioscapulohumeral Muscular Dystrophy: A Randomized
1159 Controlled Trial. *Arch Phys Med Rehabil*. 2009;90(7):1094–101.
- 1160
- 1161