Comparison of ambulatory capacity and disease progression of Duchenne muscular dystrophy subjects enrolled in the drisapersen DMD114673 study with a matched natural history cohort of subjects on daily corticosteroids

Nathalie Goemans [a],*, Mar Tulinius [b], Anna-Karin Kroksmark [b], Rosamund Wilson [c], Marleen van den Hauwe [a], Giles Campion [d]

[a] *Department of Paediatrics and Child Neurology, University Hospitals Leuven, Leuven, Belgium*
[b] *The Queen Silvia Children's Hospital, Gothenburg, Sweden*
[c] *Spica Consultants Ltd, Marlborough, United Kingdom*
[d] *BioMarin Nederland BV, The Netherlands*

Abstract

Duchenne muscular dystrophy is a rare genetic disorder with life-limiting pathology. Drisapersen induces exon 51 skipping, thereby producing a shorter but functional dystrophin protein. The longest available data are from an open-label extension study (PRO051-02) treating 12 boys with drisapersen (6 mg/kg/week subcutaneously). The median change (range) from baseline to week 177 in six-minute walking distance (6MWD) was 8 (–263, 163) metres. The current analysis aimed to put the results from PRO051-02 in the context of natural progression by comparing the functional trajectory of drisapersen-treated subjects to a matched natural history (NH) cohort, treated by standard of care. Subjects were matched individually by age and 6MWD, as the primary analysis, and by age and rise from floor (RFF), as sensitivity analysis. A total of 75 NH subjects were available for 6MWD analysis, of which matching was possible for 9 ambulant drisapersen-treated subjects. None of the 6 "stable" (baseline 6MWD ≥330 metres) drisapersen-treated subjects lost ambulation vs 4 out of 10 matched NH subjects over a comparable timeframe (~3.4 years), compared with 2 out of 3 ambulant "in decline" drisapersen-treated subjects vs all 6 matched NH subjects. A total of 79 NH subjects were available for RFF analysis. For continuous ambulatory subjects (N = 4), the RFF decline was more pronounced in the NH cohort than in the drisapersen-treated subjects. In conclusion, a comparison of ambulant drisapersen-treated subjects with matched NH subjects showed a difference in functional trajectories over a timeframe of up to 3.4 years in favour of drisapersen.
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1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked degenerative disorder and is the most common neuromuscular disease in children, affecting up to 1 in 3500 to 5000 new-born boys [1–3]. DMD is caused by mutations in the dystrophin gene including point mutations (~25%), deletions (~67%) or duplications (~7%) of one or more exons [4].

Most boys are diagnosed by the age of 5, by which time their physical ability diverges markedly from that of their non-DMD peers [5]. Untreated, muscle strength deteriorates and boys require the use of a wheelchair before reaching teenage years [5]. In addition, respiratory, orthopaedic and cardiac complications emerge, and without intervention the mean age of death is around 19 years [5]. Glucocorticosteroid treatment and respiratory, cardiac, orthopaedic and rehabilitative interventions have led to improvements in function, quality of life, health and longevity [5]. However, even with current state-of-the-art medical care, most patients do not survive beyond their third decade.

Drisapersen (PRO051/GSK2402968) is an antisense oligonucleotide inducing specific skipping of exon 51 during pre-mRNA splicing and allows synthesis of a shorter largely functional dystrophin in DMD patients. It is estimated that 13% of boys diagnosed harbour a mutation suitable for exon 51 skipping [4]. The drisapersen clinical programme involved

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more than 300 boys with skip 51 amenable DMD mutations treated for a total of over 490 patient-years.

The longest follow-up data are available from study DMD114673 (further referred to as the 673 study; PRO051-02; NCT01910649). This is an uncontrolled open-label extension of a Phase I/IIa, dose-escalation study [6] investigating the long-term efficacy and safety of subcutaneous drisapersen (6 mg/kg/week) in 12 boys with DMD, with the last efficacy assessment available at 177 weeks or 3.4 years [7]. Although a median improvement from baseline in six-minute walking distance (6MWD) of 8.0 metres (range: −263 to 163 metres) was observed following 177 weeks of treatment with drisapersen, a wide range of responses across subjects was observed, depending on ambulatory status at baseline [7].

The aim of the current study was to provide context to these results (in the light of the natural decline in ambulation expected to be seen over time in DMD patients [8–13]) and to establish the functional trajectory of these drisapersen-treated subjects from the 673 study by comparing them to a matched population from a natural history (NH) cohort, treated according to standard of care. Therefore, an NH population treated with daily corticosteroids from the Leuven Neuromuscular Reference Centre (NMRC) was evaluated to determine whether this may provide a suitable indirect comparator arm by matching characteristics of this NH population to the baseline characteristics of the drisapersen-treated subjects.

2. Patients and methods

2.1. Participants

This report documents outcome measures collected as part of an observational study of routine follow-up of DMD boys attending Leuven NMRC (forming the NH cohort) and 12

drisapersen-treated subjects from the 673 study performed at two centres (University Hospitals Leuven, Belgium, and The Queen Silvia Children's Hospital, Gothenburg, Sweden) [7]. The observational NH study was approved by the institutional review board of the University Hospitals Leuven, whereas the extension study was approved by the institutional review boards of Leuven and Gothenburg. Written consent was obtained from parents of all treated and untreated DMD boys to report their clinical assessment data anonymously.

The 673 study is an uncontrolled, open-label extension of the Phase I/IIa, open-label, dose-escalation study (Fig. 1) [6]. Subjects (N = 12) were originally dosed for five weeks in groups of three at 0.5, 2.0, 4.0, and 6.0 mg/kg/week of drisapersen, with a subsequent 13-week follow-up period [6]. Following this dose-escalation phase, and after a break of 6–15 months, all subjects entered the extension study, during which they were treated with drisapersen (6.0 mg/kg/week) for 72 weeks. A break in dosing was implemented for eight weeks, and recommenced at week 81 with an intermittent regimen (repeated cycles of 8 weeks of 6 mg/kg/week, 4 weeks off-treatment). The start of the extension study was defined as "baseline" (week 0) and the last efficacy assessment was performed at week 177 (data cut-off date: October 2014). All included subjects were genetically confirmed with DMD, had a mutation suitable for exon 51 skipping therapy and received continuous corticosteroid treatment [7].

The NH cohort data were obtained through an observational, single-centre study starting in January 2007 and recording functional time tests, age, weight, height and medication use as part of routine follow-up from genetically confirmed and corticosteroid-treated DMD boys (data cut-off date: May 2014). All functional assessments were performed by two experienced physiotherapists also participating as evaluators for eight of the
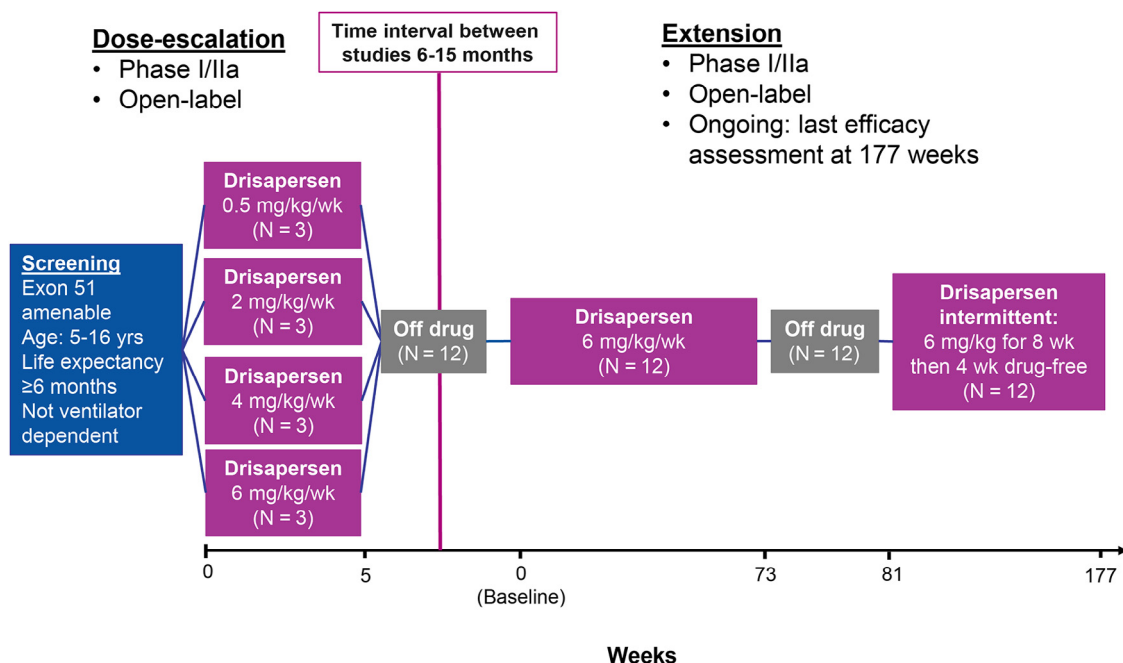

Fig. 1. Design of the DMD114673 study.

12 subjects from the 673 study. All DMD subjects up to 17.5 years were assessed for eligibility. Subjects with known severe cognitive or behavioural disorders impairing compliance with the 6MWD procedure, subjects with a clinical picture of Becker muscular dystrophy and a genetic diagnosis predicting a milder phenotype such as in-frame deletions, as well as subjects who were involved in clinical trials or had participated in any trials with investigational products were excluded.

2.2. Assessments and analysis

The 673 extension study data held by BioMarin Pharmaceutical Inc. were used to provide the age and efficacy assessments for the drisapersen arm, while the database held by Leuven NMRC provided the source data for the NH cohort. Both studies required a genetically proven diagnosis of DMD and chronic treatment with corticosteroids.

Matching was performed by first identifying subjects within the NH database that matched any of the 12 subjects from the 673 study, based on baseline 6MWD and age, as a primary analysis, and baseline rise from floor (RFF) time and age, as a sensitivity analysis. The matching criteria were pre-defined and were based on published literature showing the influence of age, baseline 6MWD and RFF as reliable prognostic indicators of outcome [9,13]. Only NH boys with more than two 6MWD or RFF assessments available were included in the comparative analyses, but all identified matches are presented in the per subject figures. The 6MWD and RFF time were assessed in the same manner in both cohorts, using a previously described methodology [6,8].

For the primary analysis, NH and drisapersen-treated subjects were matched based on age (within six months) and 6MWD (within 30 metres).

For the sensitivity analysis, NH and drisapersen-treated subjects were matched by baseline age (within six months) and RFF (within 0.5 seconds). The RFF data were converted to velocities (rise/second) in the presented figures, but are also recorded as time in seconds.

The results were compared descriptively vs statistically, given that they were drawn from two different cohorts.

3. Results

3.1. Population

In the NH cohort data set held by the Leuven NMRC, a total of 89 subjects were available for matching with 12 drisapersen-treated subjects from the 673 study. A total of 83 NH subjects were available for matching after exclusion of subjects with data recorded prior to 2007 and subjects over the age of 17.5 years, due to the inconsistent method for capturing 6MWD data and low chance of being ambulant, respectively.

Both studies required a genetically proven diagnosis of DMD and chronic treatment with corticosteroids. All drisapersen-treated subjects had an exon 51 skippable mutation, whereas the NH subjects were a generalised DMD population with confirmation of individual mutations (Supplementary Table S1). Eight out of the 12 drisapersen-treated subjects were treated with deflazacort (66.7%) and four (33.3%) with

prednisone. In comparison, 90% of the subjects in the NH cohort were treated with deflazacort, whereas 10% received prednisone treatment. All subjects were on daily steroids throughout both studies, except for two subjects (S38 from the NH population and 12 from the 673 study) who switched from continuous treatment with corticosteroids to an intermittent schedule. Subject 12 received intermittent treatment for a 10-month period between weeks 60 to 105, while subject S38 was put on an intermittent regimen from the age of 10.5 years for tolerability reasons.

As 6MWD test results are influenced by baseline ambulatory status [13], drisapersen-treated subjects were retrospectively classified by their ambulatory disease status into those walking ≥330 metres and <330 metres at extension study baseline. This subdivision reflected the clinical stage of the diseases, with subjects walking ≥330 metres considered to be in "plateau stage" or "stable". All subjects who walked <330 metres were considered to be "in decline" at extension baseline, based on the judgement of the investigator, the subjects' parents and physiotherapists according to preclinical study notes and assessment.

3.2. Primary analysis (6MWD)

A total of 75 NH subjects, receiving continuous steroid treatment, were available for matching with drisapersen-treated subjects (N = 12) from the 673 study [7]. Measurements of 6MWD trajectories over time, for each drisapersen-treated subject and their NH matches (if available), are shown in Fig. 2.

A primary analysis, matching the drisapersen-treated subjects with an NH population by baseline 6MWD (within 30 metres) as well as baseline age (within six months), was performed. Table 1 describes the number of NH matches made per 673 study subject and those who were excluded due to less than two assessments available. This table also contains the baseline 6MWD and age range of the matched NH subjects over the assessment period, to put the expected functionality from that particular group of DMD subjects into context.

NH matches for age and 6MWD could be identified for 11 of the 12 drisapersen-treated subjects (Table 1). No match for subject 5 could be found, because he had atypically strong functional ability at the age of 10.3 years (extension baseline) akin to healthy peers at the age of 13.7 years (>600 metres), despite having a definite DMD diagnosis (exon 51 amenable mutation, clinical symptoms before the age of five years and muscle pathology confirming the diagnosis of DMD). After exclusion of NH matches with less than two assessments available, 10 drisapersen-treated subjects could be matched for analysis, of which nine were able to complete the 6MWD at extension study baseline. Each drisapersen-treated subject could be matched with at least one up to seven NH subjects, involving a total of 29 NH subjects.

Seven of the nine ambulant drisapersen-treated subjects showed better functional abilities compared to their matched NH population over the observational timeframe (including all available NH matches) as well as over a comparable observational timeframe (only NH matches with ~3.4 years of follow-up or losing ambulation [6MWD of 0 metres] before). In

contrast, two of the drisapersen-treated subjects (subjects 10 and 11) ambulant at extension baseline were not distinguishable from their NH subjects over the observational timeframe (Fig. 2J and 2K) or over a comparable observational timeframe (Fig. 3). At the last scheduled assessment, four of the drisapersen-treated boys still maintained a 6MWD of >500 metres (subjects 1, 2, 5 and 7), two further maintained a 6MWD of >400 metres (subjects 6 and 9), one (subject 12) maintained a 6MWD of around 300 metres and two lost ambulation (subjects 10 and 11).

Following aforementioned criteria, the drisapersen-treated subjects were divided into two categories based on their

ambulatory ability at extension baseline. Seven drisapersen-treated patients were categorised as "stable" (baseline 6MWD ≥330 metres) and five as "in decline" (baseline 6MWD <330 metres; Table 1).

For six of the seven subjects classified as "stable", NH matches with more than two assessments could be identified. All "stable" drisapersen-treated subjects with NH matches performed better in their ambulatory ability compared with their NH matches over the observational timeframe (Fig. 2) and over a comparable observational timeframe (Fig. 3). For all six drisapersen-treated subjects for whom NH matches with a comparable observational timeframe of around 3.4 years were
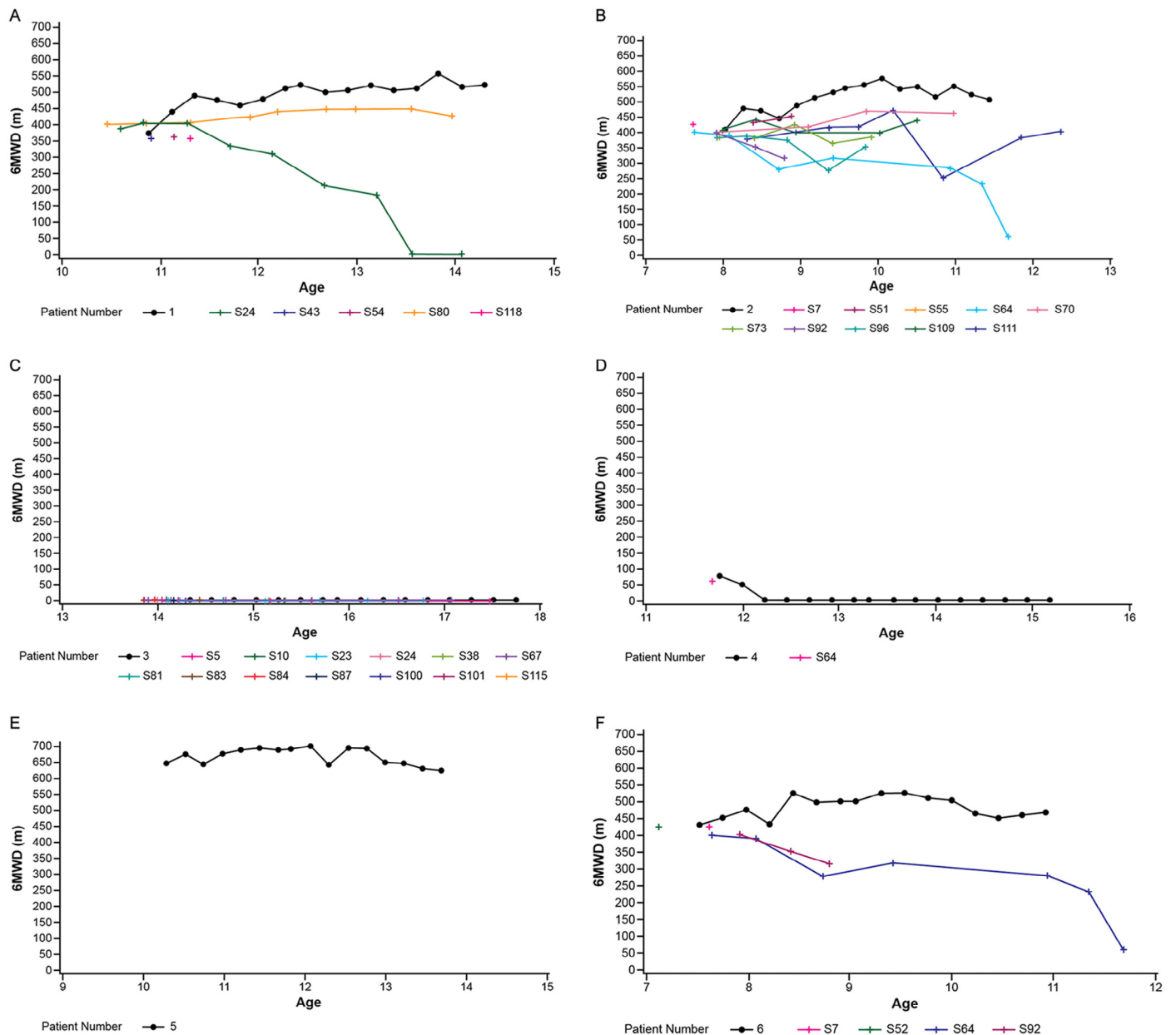

Fig. 2. Six-minute walk distance (6MWD) trajectory per matched NH subjects for PRO051-02 study subjects: (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8, (I) Subject 9, (J) Subject 10, (K) Subject 11 and (L) Subject 12. The first data-matched time point for the NH cohort was used as the control baseline. The results from the 6MWD at this baseline time point were plotted together with the results for the applicable drisapersen-treated subject over time (with time being represented as age of the boys). Although Subject 5 had DMD, his functional ability was atypically good (6MWD of 647 m) at the extension baseline when he was aged 10.3 years (continued on next page).
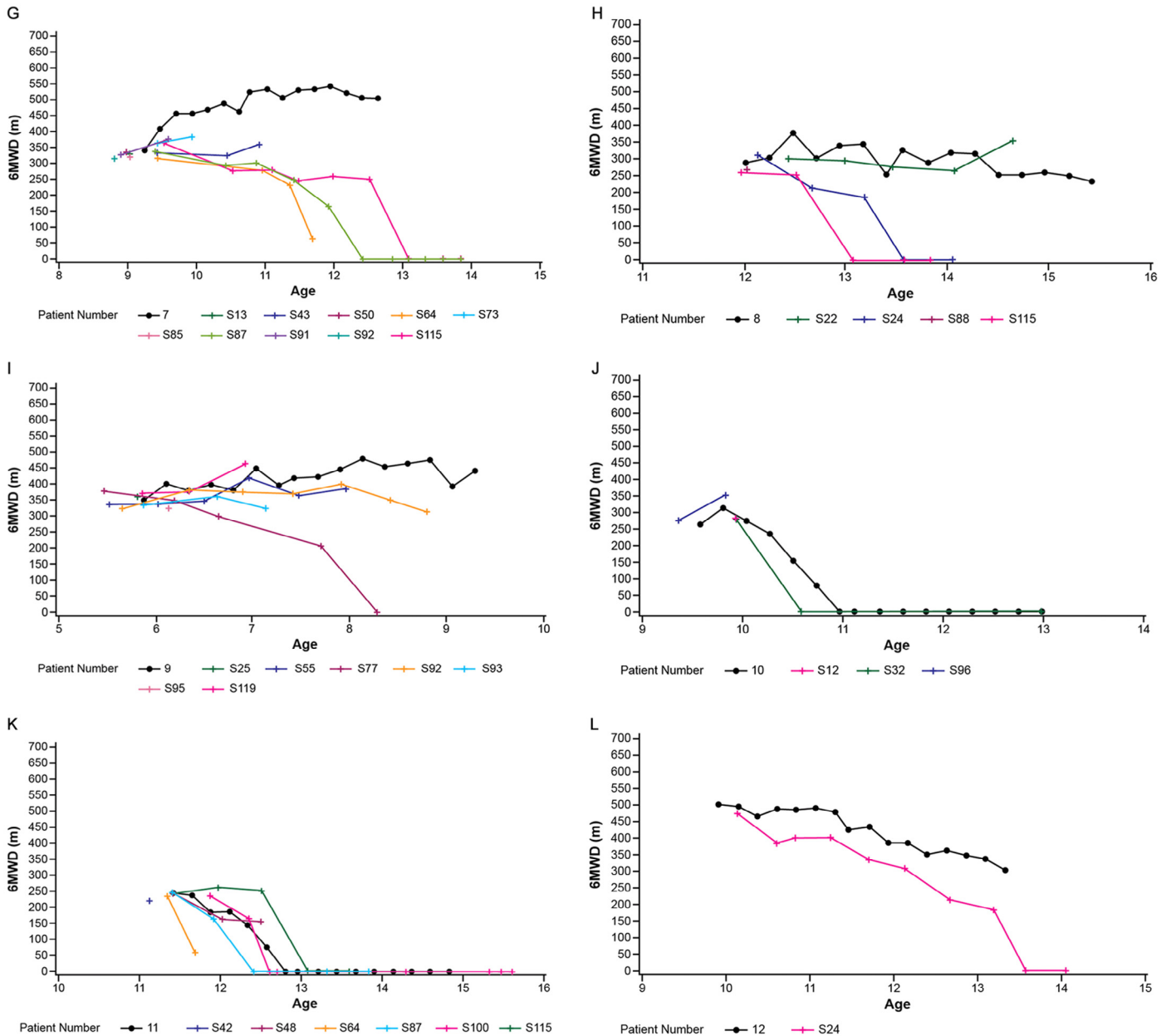
Fig. 2 (*continued*)

available, a slower decline in 6MWD (range: −198 to 163 metres) was observed compared with their NH matches (range: −475 to −58 metres; Fig. 3). Moreover, none of the "stable" drisapersen-treated subjects lost ambulation vs four out of 10 (40.0%) of the matched NH subjects over a comparable observational timeframe (Fig. 2).

Of the five drisapersen-treated subjects classified as "in decline" at baseline, NH matches with more than two assessments were available for four subjects (Table 1). Comparison of 6MWD with NH matches was possible for three of the four subjects, because one subject (3) was non-ambulant at study entry.

Of the three ambulant drisapersen-treated subjects, one subject (8) remained ambulant over the observed timeframe, whereas two of his three NH matches lost ambulation (Fig. 2H). Drisapersen-treated subject 10 lost ambulation within 1.4 years from extension

baseline, whereas his matched NH subject lost ambulation after 7.5 months. In addition, subject 11 lost ambulation in a comparable timeframe (1.4 years from extension baseline) to three out of four of his NH matches. If only NH matches who had a comparable observational timeframe of around 3.4 years or who lost ambulation (6MWD of 0 metres) before were taken into account, all 6 NH matches lost ambulation.

3.3. Secondary analysis (RFF)

A total of 79 NH subjects, receiving continuous steroid treatment, were available for matching with the 12 drisapersen-treated subjects from the 673 study [7]. RFF trajectories over time, presented as velocities (rise/second), for each drisapersen-treated subject and their NH matches (if available) are shown in Fig. 4.

Table 1
Number of natural history (NH) cohort matches per drisapersen study subject for six-minute walk distance (6MWD).

PRO051-02 study subject	NH matches identified	NH matches included*	Subject age range (years) during observation period		Baseline 6MWD range (metres)		Last assessment 6MWD (metres)		
			PRO051-02	NH	PRO051-02	NH	PRO051-02	NH	
								Median	Range
Drisapersen-treated subjects who were classified as "stable" at extension study baseline									
1	5	2	10.9–14.3	10.5–14.1	374	384–399	521	214	0–428
2	10	7	8.0–11.4	7.6–12.4	406	377–435	505	383	60–461
5[†]	0	0	10.3	No match	647	No match	625	No match	No match
6	4	2	7.5–10.9	7.6–11.7	429	400	466	187.5	60–315
7	10	4	9.2–12.6	9.4–13.9	340	317–366	503	30	0–358
9	7	5	5.9–9.3	5.5–8.8	350	322–379	441	324	0–463
12	1	1	9.9–13.3	10.2–14.1	500	475	302	0	0
Drisapersen-treated subjects who were classified as "in decline" at extension study baseline									
3	13	9	14.3–17.7	13.8–17.5	0[‡]	0[‡]	0	0	0
4	1	0	11.8–15.2	No match	75	No match	0	No match	No match
8	4	3	12.0–15.4	12.0–14.7	287	259–310	231	0	0–353
10	3	1	9.6–13.0	10.0–13.0	263	278	0	0	0
11	6	4	11.4–14.8	11.4–15.6	243	233–248	0	0	0–156

* Subjects were excluded if they did not have more than 2 assessments available.

† This subject, who had a DMD phenotype, was atypically strong (6MWD of 647 m) at the extension study baseline when he was aged 10.3 years.

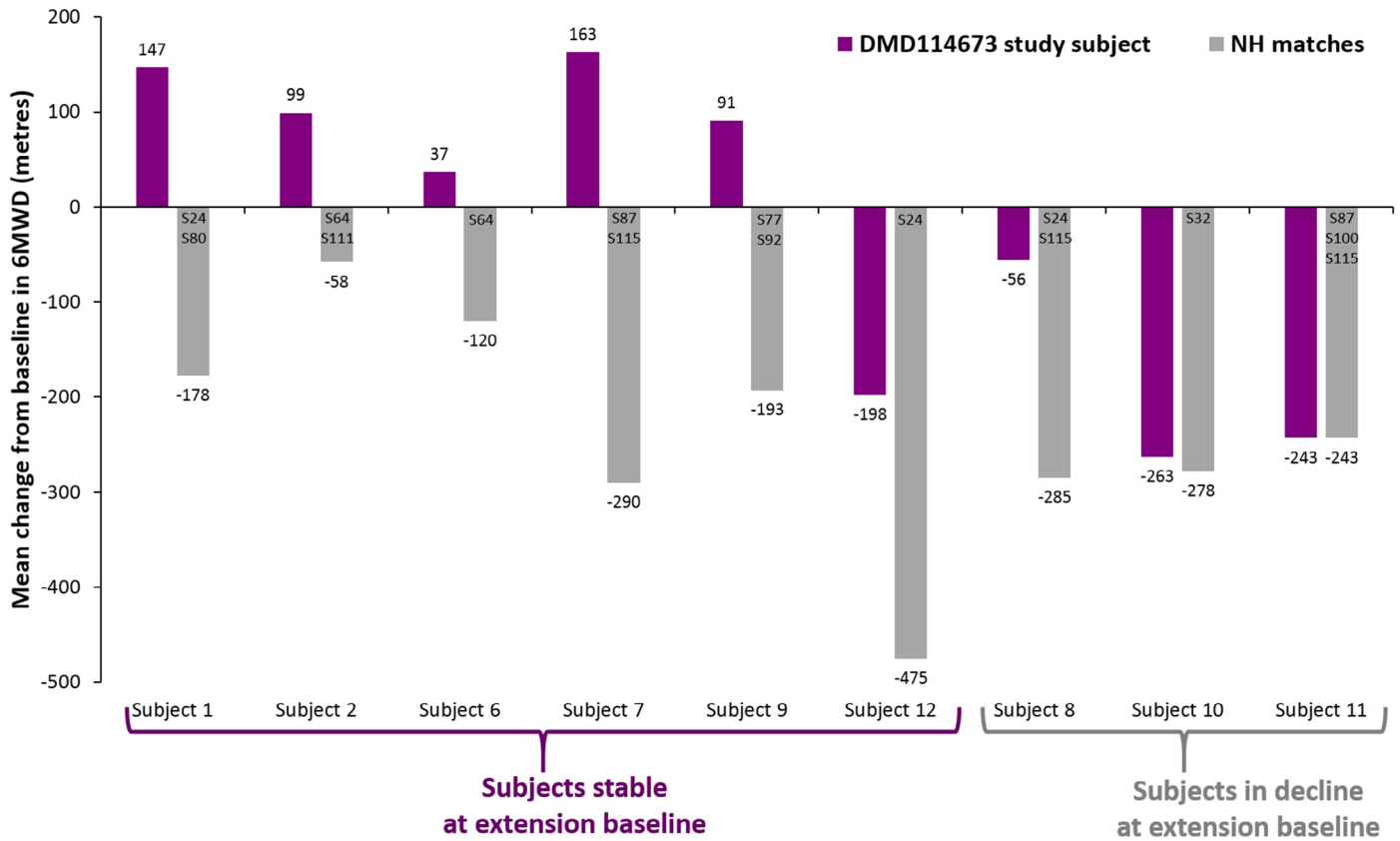‡ Not all subjects were able to complete the 6MWD test at baseline.

Fig. 3. Mean change in 6-minute walking distance (6MWD) from baseline per drisapersen-treated subject vs matched NH subject(s) over a comparable observational timeframe (~3.4 years) or losing ambulation before. Not all subjects (3 and 4) were able to complete the 6MWD test at extension baseline and one subject (5) could not be matched because, although he had a DMD phenotype, he was atypically strong (6MWD of 647 m) at the extension baseline when he was aged 10.3 years.

A sensitivity analysis, matching the drisapersen-treated subjects with an NH population by baseline RFF (within 0.5 seconds) as well as baseline age (within six months), was performed. Table 2 describes the number of NH matches made per 673 study subject and those who were excluded due to less than two assessments available. This table also presents the baseline RFF and age range of the matched NH subjects over the assessment period, to put the expected functionality from that particular group of DMD subjects into context.

Following the pre-defined criteria, NH matches for baseline age and RFF could be identified for seven of the 12 drisapersen-treated subjects (Table 2). Each drisapersen-treated

subject could be matched with at least one and up to 10 NH subjects, totalling 26 NH subjects. Three drisapersen-treated subjects (3, 4 and 11) were not matched because they had already lost the ability to perform the RFF test at study entry, while no match was possible for subject 10, who lost the ability to perform the RFF test within one year. In addition, no NH matches were identified for subject 5, as this subject's atypically strong functional ability matched healthy boys of his own age. This is consistent with the matching process for the 6MWD.

As with the 6MWD analysis, the drisapersen-treated subjects were divided into two categories based on their
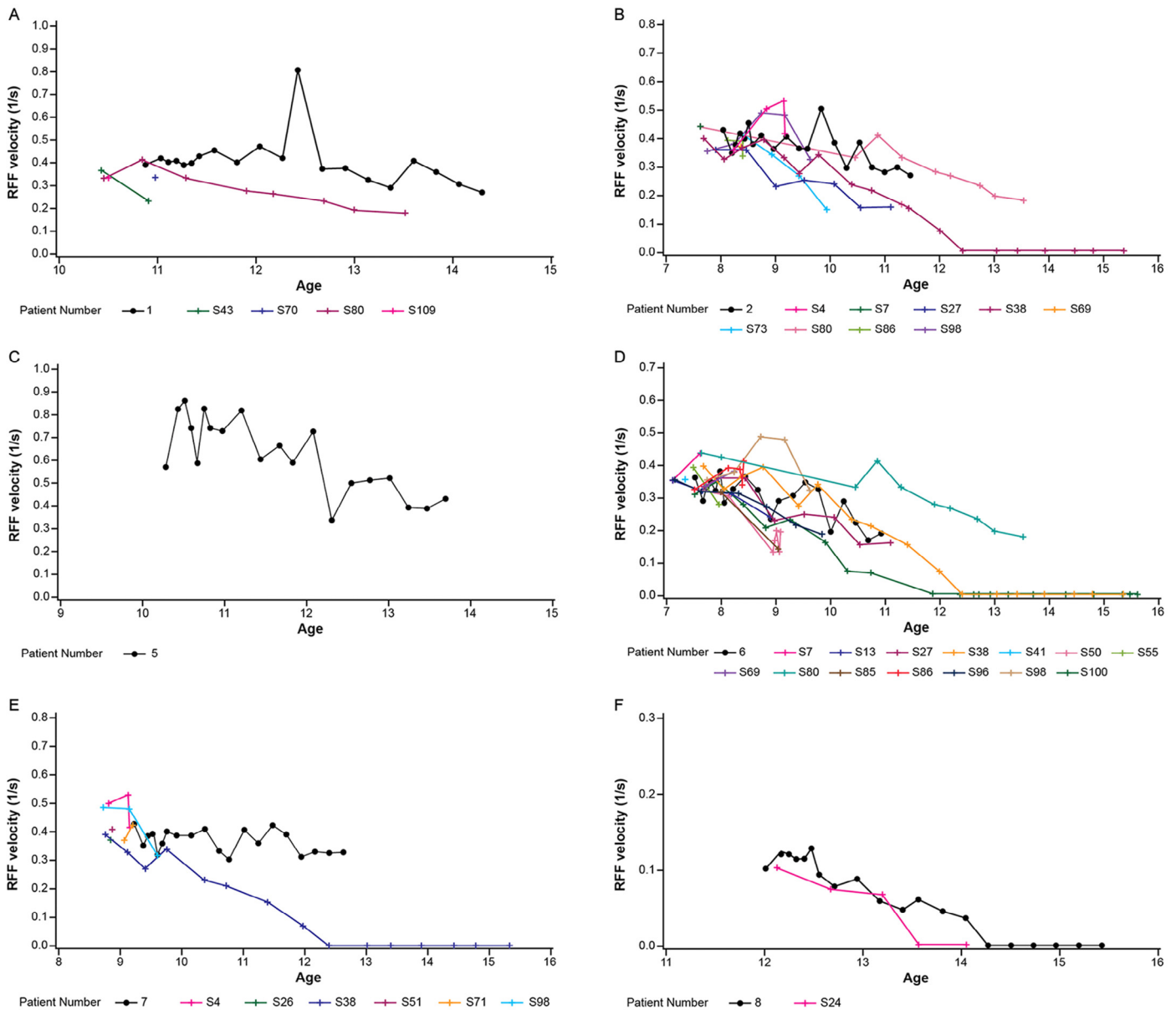

Fig. 4. Rise from floor (RFF) velocity trajectory per matched natural history (NH) subjects for PRO051-02 study subjects: (A) Subject 1, (B) Subject 2, (C) Subject 5, (D) Subject 6, (E) Subject 7, (F) Subject 8, (G) Subject 9, (H) Subject 10 and (I) Subject 12. The first data-matched time point for the NH cohort was used as the control baseline. The results at this baseline time point were plotted together with the results for the applicable drisapersen-treated subject over time (with time being represented as age of the boys). Subject 5, who had a DMD phenotype, was atypically strong (RFF of 1.75 seconds) at the extension baseline when he was aged 10.3 years (continued on next page).
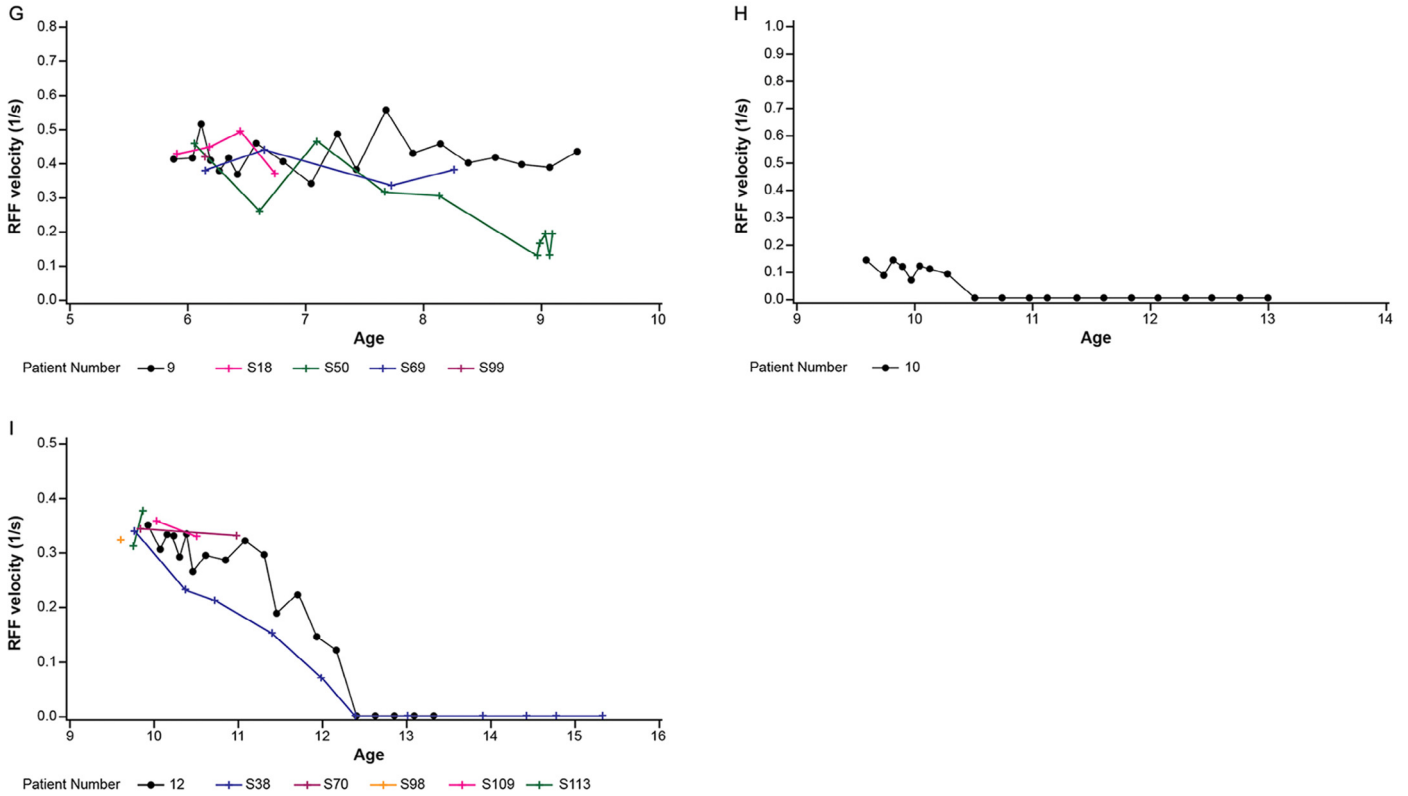
Fig. 4 (continued)

ambulatory ability at baseline. For six of the seven subjects classified as "stable" (baseline 6MWD ≥330 metres), NH matches with more than two assessments could be identified. Over the observational timeframe, five of the six matched

drisapersen-treated subjects classified as "stable" remained able to perform the RFF test compared with 20 of the 26 matched NH subjects (Fig. 4). Furthermore, after 3.4 years of follow-up, two drisapersen-treated boys still had an RFF time of <3

Table 2
Number of natural history (NH) cohort matches per drisapersen study subject for rise from floor test (RFF).

PRO051-02 study subject	NH matches identified	NH matches included*	Subject age range (years) during observation period		Baseline RFF time range (seconds)		Last assessment RFF time (seconds)		
			PRO051-02	NH	PRO051-02	NH	PRO051-02	NH	
								Median	Range
Drisapersen-treated subjects who were classified as "stable" at extension study baseline									
1	4	1	10.9–14.3	10.5–13.5	2.58	3.04	3.75	5.63	NA
2	9	7	8.0–11.4	7.7–15.3	2.35	2.31–2.84	3.75	4.49[‡]	2.42–unable[‡]
5[†]	0	0	10.3	No match	1.75	No match	2.32	No match	No match
6	14	10	7.5–10.9	7.2–15.6	2.78	2.31–3.24	5.34	5.28[‡]	7.12–unable[‡]
7	6	3	9.2–12.6	8.7–15.3	2.35	2.06–2.56	3.06	2.78[‡]	2.42–unable[‡]
9	4	3	5.9–9.3	5.9–9.1	2.44	2.19–2.66	2.30	3.09	2.63–5.15
12	5	1	9.9–13.3	9.8–15.3	2.86	2.95	Unable	Unable	NA
Drisapersen-treated subjects who were classified as "in decline" at extension study baseline									
3	0	0	14.3–17.7	No match	Unable[§]	No match	Unable	No match	No match
4	0	0	11.8–15.2	No match	Unable[§]	No match	Unable	No match	No match
8	1	1	12.0–15.4	12.1–14.1	9.83	9.81	Unable	Unable	NA
10	0	0	9.6–13.0	No match	7.2	No match	Unable	No match	No match
11	0	0	11.4–14.8	No match	Unable[§]	No match	Unable	No match	No match

* Subjects excluded if they did not have more than 2 assessments available.

† This subject, who had a DMD phenotype, was atypically strong (RFF of 1.75 seconds) at the extension baseline when he was aged 10.3 years.

‡ Not all subjects were able to perform the RFF test at last assessment; these individuals are not taken into account when calculating the median change.

§ Not all subjects were able to perform the RFF test at baseline.
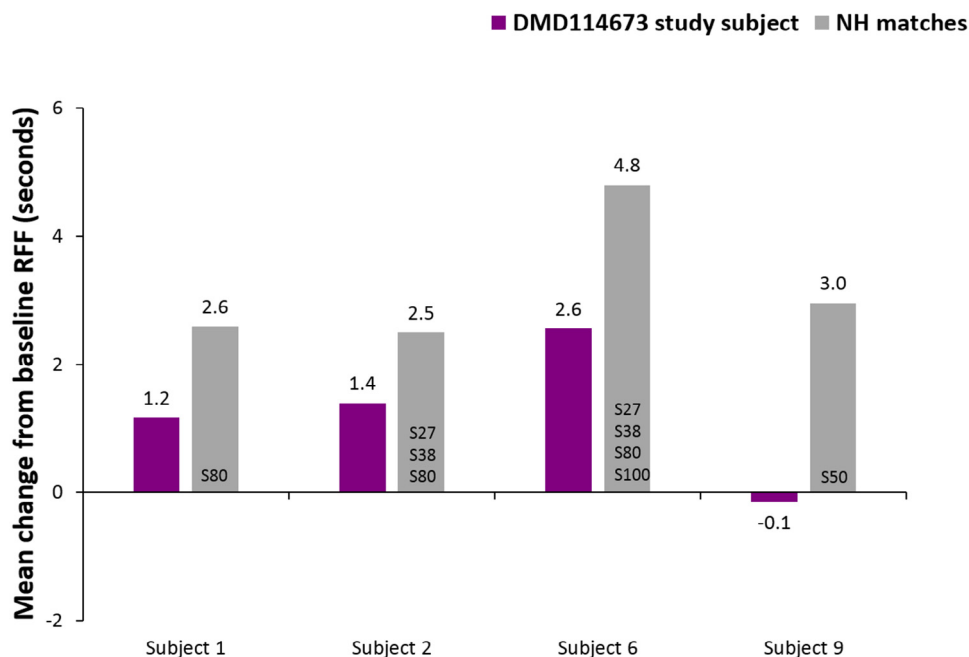
NA = not applicable.

Fig. 5. Mean change in rise from floor (RFF) from baseline per DMD114673 study subject vs matched NH subject(s) over a comparable observational timeframe (~3.4 years). Not all subjects (3, 4 and 11) were able to complete the RFF test at extension baseline and at last assessment (subjects 8, 10 and 12). One subject (5) could not be matched because, although he had a DMD phenotype, he was atypically strong (RFF of 1.75 seconds) at the extension baseline when he was aged 10.3 years. Subject 7 was not added because his NH match with a comparable observational timeframe lost the ability to perform the RFF test.

seconds (subjects 5 and 9), three further had an RFF time of <4 seconds (subjects 1, 2 and 7) and one further (subject 6) had an RFF time of <5.5 seconds (Table 2).

Five of the six matched "stable" drisapersen-treated subjects had a slower decline in RFF time as compared to their NH matches over the observational timeframe (Fig. 4), whereas one subject (12) had a comparable decline to his matched NH subject (Fig. 4I). Subject 12 lost the ability to complete the RFF test at the same age (12.4 years) as his NH match (S38). However, his NH match lost ambulation the subsequent year, while subject 12 remained ambulant at 13.3 years of age.

Five of the seven drisapersen-treated subjects classified as "stable" had at least one NH match with a comparable observational timeframe (approximately 3.4 years). For four of the five subjects, a slower increase in RFF time was observed compared with their NH matches (Fig. 5). For one subject (7), the RFF after 3.4 years of drisapersen treatment only increased by 0.71 seconds (Table 2), whereas his NH match (S38) was losing the ability to perform the RFF test (Fig. 4E).

Of the five drisapersen-treated subjects classified as "in decline" (baseline 6MWD <330 metres), three subjects (3, 4 and 11) were unable to perform the RFF test at baseline and one subject (10) could not be matched with NH controls. Subject 8, the only "in decline" subject for which NH matching was possible, had a baseline RFF time of 9.83 seconds at the age of 12.0 years and lost the ability to complete the RFF test at the age of 14.3 years (2.3 years after extension baseline). One NH match was identified, providing approximately two years of data, as described in Fig. 4G. NH subject S24 was comparable in age (12.1 years) and RFF time (9.81 seconds) as subject 8

and lost his ability to perform the RFF test at an earlier age (13.6 years). Furthermore, subject 8 was still able to walk 231 metres in six minutes at the age of 15.4 years, whereas NH subject S24 lost ambulation at the age of 13.6 years.

4. Discussion

The design and implementation of clinical studies in orphan diseases are challenging due to several factors including available population, disease heterogeneity, variations in care/management and lack of a long-term placebo control. Early clinical studies in such indications are often open-label and exploratory in design. Exon-skipping is considered a promising strategy for the treatment of DMD [14]. The 188-week open-label extension [7] of the dose-escalation study [6] of drisapersen (6 mg/kg subcutaneously) in 12 boys with DMD is the longest reported follow-up of any exon-skipping therapy. However, due to the exploratory study design, no placebo comparator arm is available. In an effort to provide context to the efficacy outcomes of this study (in the light of an anticipated natural decline in ambulation over a period of around 3.4 years in untreated subjects), we created a suitable indirect comparator arm by matching characteristics of an NH population to the baseline characteristics of the drisapersen-treated subjects.

The 6MWD test has been adapted to evaluate muscle function and endurance in neuromuscular diseases and has been chosen as the primary outcome measure in several international multi-centre clinical trials in DMD [15,16]. The decline in 6MWD is not linear over a child's life, with DMD patients who are younger than 7 years showing a lower rate of decline

compared with those older than 7 years [8–13]. In addition, baseline 6MWD values have also been shown to determine the further rate of decline, with a steeper subsequent decline observed once a baseline value of 330 metres has been reached [9–13]. Therefore, we matched the two groups for baseline age (within six months) as well as baseline 6MWD (within 30 metres).

Following the pre-defined criteria, NH matches with more than two assessments for age and 6MWD could be identified for 10 of the 12 drisapersen-treated subjects, of which nine were able to complete the 6MWD test at study entry. Seven of the nine matched drisapersen-treated subjects showed a clinical meaningful improvement (≥30 metres) [17] of functional abilities compared to their matched NH population over the observational timeframe as well as comparable timeframe, whereas two matched drisapersen-treated subjects were indistinguishable from their NH matches. There was no clear pattern on the timeframe for separation in functional trajectories between the drisapersen-treated group and the NH matches, which re-enforces the published observation of disease heterogeneity [18].

The 673 study included a clinically heterogeneous group of subjects, at different stages of disease progression. Published data indicate that patients who have a 6MWD of >330 metres have reduced risk of losing ambulation within a one- to three-year period [9,11,13]. Therefore, a sub-analysis, in which the drisapersen-treated subjects were grouped by their ambulatory disease status at extension study entry ("stable" or "in decline"), was performed.

All, except one, of the six "stable" (baseline 6MWD ≥330 metres) NH matched subjects in the 673 study were older than 7 years at the start of the extension study (mean age [range] at the extension baseline: 10.1 [5.9, 14.3] years) and would be expected to show a declining 6MWD during the study period based on longitudinal observations [9,11,13]. However, five of the six matched drisapersen-treated subjects improved in their 6MWD after 3.4 years of follow-up. In contrast, as expected, a decline in mean change from baseline 6MWD was observed in the age- and 6MWD-matched NH population. Furthermore, none of the drisapersen-treated subjects classified as "stable" lost ambulation compared with four out of 10 (40%) of their matched NH population over a comparable observational timeframe. In contrast, after the observed timeframe, two out of three (67%) of the matched drisapersen-treated subjects classified as "in decline" (baseline 6MWD <330 metres) lost ambulation compared with six out of eight (75%) of their NH matches. One subject with baseline 6MWD <330 metres remained ambulant, with a clinically meaningful [17] slower decline in 6MWD compared with the majority of his NH matches.

This difference in trajectories depending on the stage of disease (baseline 6MWD <330 metres or ≥330 metres) may indicate that a critical amount of residual muscle fibre is required to prevent further loss of ambulation with this therapeutic strategy. This theory is supported by observations in a DMD mouse model receiving exon-skipping therapy (morpholino oligomers) [19].

The RFF test can be an early predictive indicator of ambulatory loss in DMD and lack of capability of the child to perform this test precedes loss of walking ability [20]. Following the pre-defined criteria, NH matches for baseline age and RFF could be identified for seven of the 12 drisapersen-treated subjects. The four subjects who were not matched due to the inability to perform the RFF test, at extension baseline or within the year after study entry, were all categorised in the "in decline" group (baseline 6MWD >330 metres) and lost ambulation in the 6MWD review. The only "in decline" subject for which NH matching was possible lost the ability to complete the RFF test at a later age than his matched NH subject and remained ambulant at the last assessment, whereas his NH match lost ambulation.

For six of the seven "stable" subjects (baseline 6MWD ≤330 metres), NH matches with more than two assessments could be defined. After 3.4 years of follow-up, all "stable" subjects had an RFF time of <5.5 seconds compared with 13 out of 25 (52%) of their NH matches over the observed timeframe. If only NH matches with similar observational timeframes (approximately 3.4 years of follow-up) were taken into account, only one out of nine (11%) of the NH matches had an RFF time of <5.5 seconds. In addition, the increase in RFF time was smaller in the 4 drisapersen-treated subjects than in their NH matches.

While our study provides important long-term data on drisapersen, its limitations must be acknowledged. The current analysis compared NH data captured under an observational clinical study with data from an uncontrolled interventional study. However, the data from the NH comparator arm were collected in one of the two sites participating in the 673 study, ensuring consistency in standard of care and assessments between the two cohorts. A major limitation, considering the well-known heterogeneity in disease evolution, was the small numbers of subjects included in each cohort. However, by matching the two cohorts for baseline age, as well as baseline 6MWD, the most appropriate matched historical control subset was selected for comparison to the small, clinically heterogeneous cohort of the long-term open-label drisapersen (673) study.

In conclusion, comparison of ambulant drisapersen-treated subjects (from the 673 study) with matched NH subjects showed a difference in functional trajectories over a timeframe of up to 3.4 years in favour of drisapersen treatment. Despite the encouraging results in this small open-label study and in two randomised placebo-controlled phase II studies [15], McDonald C, submitted], a large global randomised placebo-controlled phase III study failed to meet its primary endpoint [unpublished data]. Further clinical development of drisapersen has been stopped as data indicated the need for improvement in the risk–benefit profile for this compound. Second generation 2′-*O*-methyl antisense oligonucleotides are currently under development.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.nmd.2016.11.013.

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