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Committee for Medicinal Products for Human Use (CHMP)

Qualification Opinion for Stride velocity 95th centile as primary endpoint in studies in ambulatory Duchenne Muscular Dystrophy studies

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QUALIFICATION OPINION as agreed by CHMP

Based on data provided by the applicant the CHMP considers that for ambulant Duchenne Muscular Dystrophy (DMD) patients 4 years of age and above, the Stride velocity 95th centile (SV95C) qualifies as primary endpoint in superiority studies as alternative to the 6 Minute Walking Test (6MWT, also called 6 Minute Walking Distance, 6MWD) provided this outcome measure is supported by consistent findings in established efficacy endpoints included as secondary endpoints.

For a detailed discussion of the CHMP assessment, please see section 4 (page 162). For the background information as submitted by the Applicant see sections 2 and 3 (pages 3-161).

The advantages of the SV95C as indicator of ambulatory function are apparent: the SV95C allows a continuous monitoring over relatively long periods in a home-setting and is therefore less sensitive to timing of the assessment (e.g. day and time of test) and relies less on patient motivation or subjective assessment as compared to established tests.

The SV95C is highly correlated to the 6MWT and is more sensitive as compared to the 6MWT, the currently most used primary endpoint in studies in ambulatory DMD. As such, the SV95C may be considered an alternative endpoint to the 6MWT in studies in DMD. The potential interchangeability between the SV95C and 6MWT is the main argument in favour of the SV95C as alternative primary endpoint in DMD studies.

The acceptance of SV95C is based on its high correlation with the 6MWT, i.e. the SV95C is an alternative to the 6MWT. For the 6MWT it is required that results are supported by consistent findings in the secondary endpoints. This also applies for the SV95C.

Acceptance of the SV95C variable is device agnostic provided accuracy and reliability of measurement are established (using a digital and passive wearable device and system[3]).

[3] All data included in the present qualification package have been recorded with the ActiMyo® device.

Nevertheless, to answer the question of the comparability between ActiMyo® and the new and smaller device, Syde®, both systems are composed of 3 parts fulfilling different sequential functions:

1) The data acquisition system, which is itself subdivided into 2 parts:
   - Two recording wearables worn by the participant, including identical sensors (accelerometers, gyrometers, magnetometers, barometer) carefully calibrated after production and regularly throughout the lifetime of the device.
   - A docking station, located in the participant's home, that retrieves data from the sensors, recharges the batteries of the wearables, and transmits the data to the software platform.
2) The software platform for data storage and monitoring by a project manager.
3) Analysis software for computing statistical variables on the recordings by a trained analyst.

The core part of the system is the data acquisition, i.e., the sensors contained in the recording wearables. The new device, Syde®, contains same or identical references as ActiMyo® for the core analog sensors. A large supply with a long-term storage is ensured by Sysnav to provide the exact continuity of the performance in all production batches.

The design in a smaller package has been studied to answer some appearance concerns of patients using the device and to be able to equip younger participants. It benefits from latest digital electronic progresses to reduce both consumption and size with a huge effort of integration.

Regarding the conformity to be used in clinical trials, Syde® functions the same way and is compliant to similar norms and standards as ActiMyo®. The declaration of conformity has been added in Appendix 7.4.

EXECUTIVE SUMMARY as provided by the Applicant

The Applicant requests qualification of the 95th centile of the stride velocity as 'essential' primary endpoint in clinical trials in ambulant patients with Duchenne Muscular Dystrophy (DMD)i.e. "*For essentially Primary Endpoint Qualification of SV95C in ambulant patients living with Duchenne muscular dystrophy (DMD) "*

The 95th centile of the stride velocity (SV95C) is a clinical outcome assessment (COA) captured by using a digital and passive wearable device and system that was developed by the Applicant. The SV95C represents the maximal speed of subject's strides performed in a real-life setting, i.e., 95% of the strides performed by the subject are slower than the SV95C and only 5% of the strides performed are faster.

SV95C has been previously qualified by the CHMP for use as secondary endpoint in DMD (EMA/CHMP/SAWP/178058/ 2019) i.e.

"*Based on data provided by the applicant and State of the art science in the field, the CHMP considers that for ambulant Duchenne Muscular Dystrophy (DMD) patients 5 years of age and above:*

- *Stride velocity 95th centile (SV95C) is an acceptable secondary endpoint in pivotal or exploratory drug therapeutic studies for regulatory purposes when measured by a valid and suitable wearable device to quantify a patient's ambulation ability directly and reliably in a continuous manner in a home environment and as an indicator of maximal performance.*
- *Stride velocity 95th centile may also be used to quantify a patient's baseline performance in such studies.*
- *Regarding use as primary endpoint for pivotal trials in this setting, although promising, more robust data gained with additional patients and longer follow-up could be beneficial: thus strengthening the long term correlation of SV95C with functional tests, expanding normative data and further supporting the justification of the clinical relevance of the proposed MCID in the PEP setting is recommended"*

Simultaneously a qualification as *"As secondary endpoint for other progressive neuromuscular diseases characterized by proximal muscle weakness"* has been initiated. This is <u>not</u> part of this current Qualification Opinion statement.

The purpose of this request is, first, to seek qualification of the SV95C for use as a primary endpoint in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity in real-life of ambulant patients with DMD. Additional evidence which builds upon that presented previously in the secondary endpoint qualification opinion package are presented to address the comments raised by the CHMP at that time. Second, the data package includes also SV95C qualification data for other diseases characterized by proximal muscle weakness.

In addition, the data processing chain (from the reconstruction of the movement to the clinical variable) is managed in configuration and therefore that any evolution is traced and is followed by a verification/validation step to ensure that the deviations are in the order of magnitude of the numerical errors of the calculators and chain of compilations and therefore several orders of magnitude below the LSB for the application.

BACKGROUND as submitted by the Applicant

1. Executive Summary

1.1. The Objective of the Request

Duchenne muscular dystrophy (DMD) is the most frequent dystrophy in childhood characterized by a proximal muscle weakness leading to progressive difficulties in ambulation and ultimately in loss of walking ability.

The 6-minute walk test (6MWT; an exercise test that measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes) has over recent years been considered the gold standard evaluation in DMD trials[1,2] that are focused therapeutically on preservation of ambulation (e.g., NCT02255552, NCT03179631). Other outcomes, such as the 4-stair climb test (4SC),[3] or the North Star Ambulatory Assessment (NSAA) scale,[4] have also been utilized as primary outcome in ambulant DMD patients (e.g., NCT02851797, NCT04281485, NCT03039686, NCT05096221). However, all these assessments only provide a snapshot overview of the supposed maximal patients' functional ability. Wearable technology offers the opportunity to assess patients in real life and provide continuous assessment that integrates patients' day to day fluctuation. The 95th centile of the stride velocity (SV95C; a clinical outcome assessment (COA) captured using a digital and passive wearable device and system[4]) was developed and previously qualified by the Committee for Medicinal Products for Human Use (CHMP) as an acceptable secondary endpoint for use in pivotal/exploratory therapeutic studies in DMD (European Medicines Agency (EMA)/CHMP/Scientific Advice Working Party (SAWP)/ 178058/2019).

The purpose of this request is, first, to seek qualification of the SV95C for use as a primary endpoint in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity in real-life of ambulant patients with DMD. Feedback received from the CHMP during the qualification process as a secondary endpoint indicated that more robust data with additional patients and longer-follow up were recommended for consideration as a primary endpoint, and that some data demonstrating the sensitivity to positive change should also be provided. Therefore, in this follow-up qualification package, additional evidence which builds upon that presented previously in the secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019) are presented to address the comments raised by the CHMP at that time, to confirm and further inform the different measurement properties of the SV95C, and to support its use as a primary endpoint to assess new drug efficacy in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity of patients with DMD. This includes evidence of content validity from qualitative research (refer to Section 3.2.1)

[4] The recording device and accompanying system used two watch-like sensors - each containing tri-axial accelerometer, gyrometer, magnetometer(s) and barometer that record the linear acceleration, the angular velocity, the magnetic field of the movement in all directions and the barometric altitude – as well as one docking station. For ambulant patients one device is placed near the ankle and the other is placed on the second ankle or worn as a wristwatch. The device should be able to detect all strides at all paces (slow to fast and turning strides). The segmentation of the start and end of a stride is based on a model linking the ankle acceleration and angular velocity on the principle that the lower limb is in rotation around the heel. The length and velocity of the strides should be accurately measured with an error at 1 sigma (68% CI) under 2.5%. Tests for security (EN 60601-1:2007 professional healthcare and EN 60601-1-11:2015 at home), electromagnetic compatibility (IEC 60601-1-2:2014 professional healthcare and IEC 60601-1-2:2014 at home), biocompatibility (ISO 10993-1:2009) and usability (IEC 60601-1-6:2010 and IEC 62366-1:2015) for CE marking are associated. Software development follows EC 62304. Communication channels are encrypted (SSH, HTTPS) - Only the researcher has access to a patient identifier code that indicates that a device has been used by the same patient in a certain recording period. But the link between a patient identifier code and the personal details is only stored by the clinical center together with the clinical and medical information. Data are stored in an internal memory inside each watch-like device and transferred to the docking station, every night, when they are put to charge. Data collected in the docking station can be sent anonymously directly via Internet on a dedicated and secure web-cloud or can be stored on an internal USB drive for up to 3 months. Computation of variables is performed afterwards for each patient using the recorded magnetoinertial data. Recording does not rely on individual patient calibration and contrary to optical motion capture systems it can be used continuously, including in the home environment.

and additional quantitative data [test-retest reliability, construct validity, responsiveness, and meaningful change threshold (MCT) analyses (refer to Section 3.2.2)]. Data was also provided on patients who are just initiating steroid treatment.

In addition, several clinical developments are ongoing and planned on other neuromuscular diseases (NMDs) characterized by a proximal muscle weakness leading to progressive difficulties in ambulation such as spinal muscular atrophy (SMA) Type 3, centronuclear myopathy (CNM), limb girdle muscular dystrophy (LGMD), or facioscapulohumeral muscular dystrophy (FSHD), using same or similar COA as for DMD clinical trials (i.e., 6-minute walking distance [6MWD], modified NSAA) with similar limitations (NCT03056144, NCT04915846, NCT04003974, NCT03783923). Due to the rarity of those diseases, increasing the sample size to allow an acceptable power in clinical trials is challenging. Therefore, we included in this application some evidence from other progressive NMDs characterized by proximal muscle weakness, SMA Type 3, CNM, LGMD, and FSHD. The very similar findings in this group of disease not only provides further evidence to support the DMD primary endpoint application (refer to Section 4.2) but also support the extension of qualification of SV95C for these other rare and very rare NMD disorders where proximal motor function is recognized as the primary marker of disease progression.

1.2. The Need for and Impact of SV95C in Clinical Drug Development

As was outlined in the summary section (pages 14 and 15) of the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019, DMD is a rare and devastating childhood disease, affecting 1 in 5,000. The earliest symptoms of progressive muscle weakness (e.g., pain, fatigue, inability to walk as fast as their peers, inability to walk long distance) impact the ambulatory ability in patients with DMD.

The number of potentially effective therapeutic approaches in DMD are increasing,[5] and thus, the demand for validated COA measures to demonstrate a clinically meaningful therapeutic response over time in clinical trials is higher than ever. Several primary endpoints have been used to try to demonstrate a positive treatment effect in clinical trials targeting ambulant patients with DMD: changes in the maximal distance walked in 6 minutes (6MWD), estimated by the 6MWT[1], in the Motor Function Measure (MFM) that includes 32 items and provides a score on a 0 to 96 scale,[6,7] in the NSAA that includes 17 items noted on a 0 to 2 scale and that provides a 0 to 34 point score, and in other timed-function tests such as the time to climb 4 stairs (4SC), or the time to Rise from Floor. These functional tests present with a major limitation: they provide only a glimpse of what is assumed to be the patient's maximal functional ability the day of the assessment, based upon an assessment performed in a clinic setting. Patients with NMDs typically present with good and bad days, can present with intercurrent illness the day of the assessment, and can be tired because of the travel between the domicile and the investigation site. In addition, these assessments are time consuming- they require the patient to travel to the investigation site, which has caused major protocol deviations during the pandemic, and are also partially subjective as they can vary based on the evaluator.[8]

SV95C is a measure that addresses the issues with the existing COAs described above. It is a digital COA based on a wearable device and system that passively collects data. SV95C presents a significant advantage over the classic 6MWT or other functional scales as it provides continuous monitoring over relatively long period in a real-world setting and hence is less sensitive to bias on the day of clinic visit and does not rely on patient motivation or subjective assessment. It is thus more representative of the patient's real ambulatory capabilities. Using a wearable device and system is likely to also overcome variations in practice encountered across different centers/countries, which also has a significant impact on the reliability of results, particularly in global studies. Assessment of ambulatory capabilities in daily life using a wearable device therefore offers a much more clinically relevant and powerful outcome measure to demonstrate efficacy in DMD clinical trials. As a home-based measure it also

helps alleviate some of the demands induced by travel and the family organization which is necessary for the site-based visits required by existing tests, reducing the burden on both patients and sites in clinical trials.

1.3. SV95C Characteristics

SV95C is a COA that is derived from a digital and passive data collection device that was developed based on magneto-inertial technology that aims to measure the maximal stride velocity of patients living with DMD. SV95C was selected as the most sensitive to change and highly reliable variable derived from the wearable device[a] used, among a list of outcomes established by physicians specialized in DMD follow up and clinical trials. It represents the maximal speed of the subject's strides performed in a real-world setting, *i.e.,* 95% of the strides performed by the subject are slower than SV95C and only 5% of the strides performed are faster.

In the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019), it was demonstrated that SV95C, when measured with a suitable wearable device[a] fixed at the ankle, is accurate, reliable, sensitive to change, and clinically relevant based on the correlations with existing COAs of established clinical relevance (6MWD, NSAA, and 4SC test; refer to Table 1 on page 13 in the previous secondary qualification opinion for a brief summary of the results and analyses). Nevertheless, regarding its use as primary endpoint, EMA stated that*, "although promising, more robust data gained with additional patients and longer follow-up could be beneficial: thus, strengthening the long-term correlation of SV95C with functional tests, expanding normative data and further supporting the justification of the clinical relevance of the proposed MCID in the PEP setting is recommended."*

Furthermore, the device[a] and algorithms remain unchanged from what was outlined in the prior application, and therefore still meets all required regulatory standards for digital devices.

1.4. Sources of Data and Major Findings

1.4.1. Qualitative Evidence (Content Validity)

Discussions were held with clinician experts and as part of the SKIP-NMD European project. Different ways to characterize ambulation were explored through gait parameters or distance walked, and several conceptual variables related to ambulation (stride length, stride speed, distance walked, etc.) were developed. Based on these discussions with clinician experts and other insights as reported in the April 2016 SKIP-NMD project report [Confidential appendix Section 7.7], it was agreed that the key area of focus was on variables related to the steps, namely stride length and stride speed.

Those variables were then tested on data collected from patients enrolled in clinical trials where variables derived from a wearable device were used as an exploratory outcome measure and clinician experts agreed that the maximal speed at which a patient is able to move around in a real-life setting was a clinically meaningful outcome.

Feedback and insights have also been obtained from the comments made by non-profit organizations, industry, and the scientific community during the public consultation of the previous SV95C EMA qualification process for use as a secondary endpoint. Online surveys (Appendices 7.1 and 7.2) were conducted of HCPs and of patients and caregivers to obtain more in-depth feedback on the importance of ambulation and the need for, and acceptability of, a wearable device to assess ambulatory capabilities, as well as the meaningfulness of measuring the maximal speed developed to move around in the real-world setting to evaluate an improvement in the patients' condition.

Overall, the survey data confirms that ambulation is a key element of DMD from the patient/caregiver and HCP perspective. Clinicians, patients, caregivers, and representatives in industry alike recognize

the need for a measure that is not restricted to the clinic setting and influenced by factors relating to this setting or dependent upon individual or environmental factors at play at the point of the test administration. From a patient/caregiver perspective, ambulation is a key aspect of DMD, one that is associated with independence or freedom and the ability to get around. A lack of ambulation leads to a reliance on technology and others, and it is the function that patients/caregivers would most like to see restored in a clinical trial. Additionally, a change in top speed of walking was considered appropriate to represent an improvement in patient's ambulatory capacities. The majority of patients/caregivers indicated a preference for a wearable device to capture mobility in a clinical trial, reporting that such a device would make participating in a clinical trial more attractive, and that they would be willing to use it for as long as the trial lasts. This feedback from the patients/caregivers in DMD was also observed in other NMDs surveyed.

1.4.2. Quantitative Evidence

Sources of data used in the present qualification opinion package are presented in Table 1 and Table 2. Only participants with at least 50 hours of recordings during a recording period are considered into the analyses. Patients considered in longitudinal analyses are patients followed at least over 3 months with at least 50 hours of recordings in each recording period. Data from clinical trials A and B (CT-A and CT-B) were used for longitudinal analyses of the natural course of the disease as no efficacy of the investigational medicinal product was shown over 12 months of follow up.[9]

The 45 patients with DMD used in the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019) are indicated in bold in Table 1

Table 1: Source of Data for the DMD and Control Populations

	NHS-A	NHS-B	NHS-C	CT-A	CT-B	CT-C	In Clinic
Number of ambulant patients with DMD							
Equipped with a wearable device	3	20	13	40	58	7	9
Used for cross sectional analyses at BL	2	13	**11**	**35**	51	7	7
[5 -7]		8	8	16	15	2	6
[8 – 14]	-	5	3	18	36	5	1
Used for longitudinal analyses- NHS	-	5	**6**	**27**	46	-	-
[5 -7]	-	2	3	14	14	-	-
[8 – 14]	-	3	3	13	32	-	-
Used for longitudinal analyses- Treatment	2	1	-	-	-	-	7
[5 -7]	2	1	-	-	-	-	6
[8 – 14]	-	-	-	-	-	-	1
Number of control subjects							
Equipped with a wearable device	91	9	-	-	-	-	-
Used for cross sectional analyses at BL and younger than 15 years	62	4	-	-	-	-	-
[6 -7]	15	2	-	-	-	-	-
[8 – 14]	47	2	-	-	-	-	-

BL = Baseline; CT = clinical trial; DMD = Duchenne muscular dystrophy; NHS = natural history study, [age range]

Table 2: Source of Data for Other Progressive NMDs with Proximal Muscle Weakness

	NHS-CNM-A	NHS-SMA-A	NHS-SMA-B	CT-FSHD-A	CT-FSHD-B
Number of ambulant NMDs patients					
Equipped with a wearable device	9	7	15	8 LGMD + 7 FSHD	14
Used for cross sectional analyses at BL	7	6	14	5 LGMD + 5 FSHD	14
[6 – 17]	2	3	5	-	-
[18 – 65]	5	3	9	5 + 5	14
Used for longitudinal analyses- NHS	6	6	-	-	-
Used for longitudinal analyses – Treatment	-	-	10	-	-

BL = Baseline; CT = clinical trial; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; NHS = natural history study, [age range]; SMA = spinal muscular atrophy

A brief summary of the key quantitative analyses and results is presented in Table 3.

Table 3: Summary of Key Quantitative Analyses and Results

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
Accuracy	• The distance walked in 6 minutes measured by physiotherapists during the 6MWT and the distance calculated with the wearable device was compared in 23 patients with DMD (31 tests analyzed). Results showed that the distances measured by physiotherapists and those computed by wearable sensors were similar (after adjusting for the distance from turning around the cones at each 25-meter corridor extremities in the 6MWT; difference: 0.75 m ± 8.9 for a mean 307.6 ± 103.5 m). • In a motion capture room, 8 healthy subjects walked based on 3 defined trajectories and at 3 different gait cadences (slow, normal, and fast). The system detected 98.7% of the strides, and at the fast-walking speed, the system measured the stride speed with high accuracy (mean = 152.88 cm/s; mean difference = 0.01 cm/s; RMS difference = 1.02 cm/s).	• No additional accuracy data are provided in the present qualification opinion package for patients with DMD. • The distance walked in 6 minutes measured by physiotherapists during the 6MWT and the distance calculated with the wearable device was compared in 4 patients with SMA (7 tests analyzed) and in 2 patients with CNM (2 tests analyzed). Results showed that the distances measured by physiotherapists and those computed by wearable sensors were similar after adjusting for the distance from turning around the cones at each 25-meter corridor extremities in the 6MWT (see p25/77 of the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019)). The differences were -1.4. ± 0.6 m for a mean distance walked in 6 minutes of 393.7 ± 72.4 m for patients with SMA and -1.4. ± 2.5 m for a mean of 280 ± 225 m for patients with CNM.	4.2.2.2-
Repeatability			

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
Test-retest Reliability	• ICC was calculated based on measures performed 15 days apart in the 1-month baseline recording period, in 45 patients with DMD. • ICC for the 95th percentile stride velocity was high (0.937), indicating excellent reliability between the 2 measures.	• Assuming no significant disease progression over 2 months, the excellent reliability of SV95C was confirmed based on measures performed 1 month apart in 2 successive recording in 52 patients with DMD (ICC = 0.970) and was verify with Bland and Altman graphical analysis. • The excellent reliability was also observed in other progressive NMD with proximal muscle weakness such as SMA (N = 6, ICC = 0.999), CNM (N = 6, ICC= 0.985), FSHD (N = 14, ICC = 0.991)	3.2.2.2 4.1.2.2.1

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
Robustness	• Using 28 patients assessed in a non-controlled setting, the relationship between the recording period average and the variability of the measure was studied by tracing the Sysnav Variance. Good stability was reported, with low variability up to 4.41% for the 95th percentile of stride velocity (based on 180 hours of wearable device and system use). • The influence of the time of recording, morning versus afternoon, weekday or weekend, on SV95C variability was also assessed on 45 and 10 patients with DMD, respectively. For the 45 patients, no significant differences were found between morning (mean 1.564 m/s and SD 0.384 m/s) and afternoon (mean 1.600 m/s and SD: 0.387 m/s) recording periods. The mean difference between morning and afternoon session was 0.036 ± 0.215 m/s). In contrary, significant differences were observed between weekdays and weekend days (mean difference = -7.34 ± 9,19%) meaning that due to probable difference in activities, it is important to ensure that data are collected on every day of the week to limit bias in the result.	• No additional data are provided on robustness in the present qualification opinion package • Using 4 SMA and 3 CNM patients assessed in a non-controlled setting, the relationship between the recording period average and the variability of the 95th percentile stride velocity was assessed. Overall, good stability was observed, with a low variability of less than 4% and 5% reported for the SMA population (based on 180 and 50 hours of wearable device and system use, respectively). Similarly, for the CNM population, a recording period of 180 hours led to a variability of less than 6% and a recording period of 50 hours led to a variability of 8%. • There was no impact of recording in the morning versus the afternoon for the SMA, CNM, LGMD, and FSHD populations, except for some patients with FSHD, and no impact of recording during the week versus the weekend for any population.	4.2.2.3.2
Construct Validity			
Known-groups Validity	• Interim analysis provided in the previous qualification package demonstrated graphically that stride velocity were clearly different in patients and controls.	• Known-groups validity was assessed by comparing SV95C of patients living with DMD	3.2.2.3.1

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		(N = 125) to SV95C measures in an aged-matched control population (N = 66). • SV95C was able to discriminate patients with DMD from the healthy control subjects, with lower median SV95C scores reported for patients in the DMD population (1.563 m/s) compared with the healthy control population (2.713 m/s; P-value < 0.001) • Similar results were observed for the 6MWD and 4SC, where the median 6MWD score was statistically significantly lower in the DMD population compared with the healthy control population. The NSAA was not performed by healthy subjects. • Known-groups validity was also assessed by comparing SV95C of youngest (5 to 7 years) to oldest (8 to 14 years) patients living with DMD. A statistical difference was observed between the youngest and oldest DMD population for both the SV95C and 4SC, indicating that DMD patients in the older population had a lower stride velocity and took longer to climb 4 stairs compared with the younger population (median SV95C: 1.39 m/s versus 1.68, respectively; median 4SC: 3.75 seconds versus 3.06 seconds). No	

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		statistical significance was observed for the 6MWD and NSAA between age groups for the DMD population. • Lastly, known-groups validity of SV95C was assessed by comparing SV95C of other progressive NMDs characterized by a proximal muscle weakness SMA (N = 20), CNM (N = 7), FSHD (N = 19), LGMD (N = 5) to the control population (N = 93). As for DMD, SV95C was able to discriminate patients with NMDs from the healthy control subjects, with lower median SV95C scores reported for patients in the SMA (1.174 m/s), CNM (1.043 m/s), FSHD (1.284m/s), or LGMD (0.533 m/s) population compared with the healthy control population (2.500 m/s; P-value < 0.001).	4.2.2.4.1
Convergent Validity	• The convergent validity of the 95th percentile of stride velocity to existing COAs (6MWD, 4SC, and NSAA) was assessed using data from 45 patients with DMD. • Moderate but significant correlations of the 95th percentile of stride velocity with the 6MWD (ρ = 0.542), the NSAA (ρ = 0.645), and the 4SC (ρ = 0.547) were observed at Baseline.	• The convergent validity of SV95C was assessed by cross-correlating SV95C to existing COAs (6MWD, 4SC, and NSAA) using data from 62 additional DMD patients with available data (107 patients total [including the 45 patients from the previous qualification opinion dossier]). • SV95C was significantly correlated with the 6MWD, NSAA and 4SC (P-values < 0.001) at Baseline, with the following Spearman	3.2.2.3.2

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		correlation coefficients 0.657, 0.644, and -0.634 respectively. • Similar results were observed at 3, 6, 9, and 12 months follow-up. • The convergent validity of SV95C was also confirmed in other progressive NMDs characterized by a proximal muscle weakness such as SMA (N = 14), CNM (N = 7), FSHD (N = 13) with a strong correlation between SV95C and 6MWD in SMA (ρ = 0.836, P-value = <0.001), CNM (ρ = 0.929, P-value = 0.003), and FSHD (ρ = 0.770, P-value = 0.002). • SV95C was also strongly correlated to the global function scale MFM total score (SMA, N = 15, ρ = 0.790, P-value = <0.001 – CNM, N = 7, ρ = 0.857, P-value= 0.014)	4.2.2.4.2
Responsiveness			

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
Ability to detect the change during the natural course of the disease	• Responsiveness, or the ability to detect change, was assessed by following 31 DMD patients over 6 months and 11 patients over 12 months. • There was a significant decline for the 95th percentile stride velocity at 6 months (-6.8% [P-value < 0.001]) and 12 months (-13.8% [P-value = 0.008]).	• Responsiveness of SV95C was determined by using the natural change over time at 3, 6, 9 and 12 months in 81, 59, 39, and 28 patients, respectively on a stable regimen of corticosteroids or having initiated corticosteroids from at least 6 months. • The ability of the SV95C to detect a negative change was established as early as 3 months. A continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.044, -0.067, -0.110, and -0.204 m/s, respectively), with statistically significant median score changes observed at each time point (P-values < 0.001). • When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for each group, indicating that the loss of the maximal speed was progressive over time. A larger decrease was observed in patients 8 to 14 years of age (median change from baseline scores ranged from -0.044 m/s at Month 3 to -0.210 m/s at Month 12) compared with patients aged 5 to 7 years (median change	3.2.2.4

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		from baseline scores ranged from -0.023 m/s at Month 3 to -0.197 m/s at Month 12). • When using the sub-populations who performed SV95C and other COAs such as 6MWD, NSAA, or 4SC, in contrary to most of existing COAs, a continual significant decline in the median change from baseline SV95C at Month 3 to Month 12 were observed. These results indicate that the SV95C may be more sensitive to detect disease progression over the course of 12 months compared with the other COAs (6MWD, NSAA, and 4SC). • Responsiveness of the SV95C was also determined from a set of 17 patients who were followed over 12 months. Despite the small sample size, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline SV95C were -0.043 m/s, 0.067 m/s, 0.157 m/s, and -0.197 m/s, respectively), with statistically significant median score changes observed at Months 6, 9, and 12 (P-values < 0.01). • A decline in SV95C over time was observed in a small population of patients with SMA (N =	

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		6) and CNM (N = 7) but changes were small and not statistically significant.	4.1.2.4
Ability to detect the change due to treatment improving the condition	• The sensitivity of SV95C to a positive change was only approached visually with 2 DMD patients who were started on corticosteroids.	• The sensitivity of SV95C to a positive change was assessed in 11 patients with DMD who were started on corticosteroids. • A significant positive change in SV95C as early as 3 months was observed (P-value = 0.003), which would indicate an improvement in response to treatment. This was confirmed at 6 months based on the median SV95C change scores from Month 3 to Month 6 (0.0901 m/s and 0.211 m/s, respectively). • The sensitivity of SV95C to a positive change was also assessed in 10 patients with SMA who were started on Nusinersen. No significant changes were observed over time but in	3.2.2.4.4 4.2.2.5.3

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		contrast to untreated patients with SMA, we did not observe any trend of decline in SV95C.	
Meaningful Change Thresholds (MCTs)			
Distribution-based threshold	• The minimal clinically important difference (MCID) was statistically measured based on the standard error of measurement (SEM) in 40 patients with DMD. The MCID was calculated using the baseline SD and the ICC calculated on the first 15 first to the last 15 days in a 1 month recording period. A relative MCID was given by dividing the MCID by the mean of the variables at Baseline. • The MCID (relative MCID) was found to be 0.0985 m/s (6.24%).	• The SEM and the minimal detectable change (MDC) of SV95C at 80%, 90% and 95% confidence levels were calculated (N = 103). • The SEM was calculated as 0.070 m/s for the DMD population in patients aged 5 to 14 years. The MDC of SV95C at the 80%, 90% and 95% confidence level were 0.127 m/s, 0.163 m/s, and 0.194 m/s respectively. • Similar results were observed when the age group was stratified by younger and older populations.	3.1.2.5.1
Anchor-based within patient threshold	• Anchor-based within patient thresholds were not calculated in the previous qualification package.	• The MCT of SV95C was assessed through an anchor-based approach based on the PODCI subdomain "transfers and basic mobility" and CGI-C scales performed at Week 48 in a subgroup of 6 to 11 years of age DMD patients enrolled in a clinical trial prematurely stopped due to lack of efficacy. PODCI (N = 15) were completed by parents. Subscores are expressed in percentage, highest score meaning no limitation. CGI-C (N = 12) was completed by clinician. It is a qualitative scale	3.2.2.5.2

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		coded from 1 = very much improved to 7 = very much worsened. • A significant correlation was observed between the SV95C and the CGI-C (Spearman coefficient correlation ρ = -0.816, P-value = 0.001). Similarly, a correlation between the SV95C and the PODCI "transfers and basic mobility" subscore was observed (Spearman coefficient correlation ρ = 0.611, P-value = 0.015), but no correlation was found between the changes from Baseline. • Despite the lack of IMP efficacy, most participants reported a stabilization or improvement. Only a few participants recognized a worsening after 48 weeks of follow up. Median SV95C change scores in patients reported to have worsened on the CGI-C were -0.280 m/s and on the PODCI -0.245 m/s. • Interestingly, the difference between the median SV95C change to consider a subject stable or worsened was -0.145m/s and -0.07m/s considering PODCI and CGI-C respectively	

SV95C properties	Results from the Previous Secondary Endpoint Qualification Opinion Package (EMA/CHMP/SAWP/178058/2019)	Results Presented in the Current Qualification Opinion Package	Corresponding Section Number
		• These values suggest that an anchor based MCT of between -0.1 and -0.3 could be taken to indicate a meaningful change.	
Overall estimates of MC	• No estimates were provided	• An estimate of -0.10 for MCT is suggested. This value is consistent with the anchor-based change score in those patients considered to have worsened and is also larger than the estimate of measurement error. It is also supported by the decline observed in the natural course of the disease and the improvement after starting corticoids	3.2.2.5.3
Generalization to Other NMDs	• Data (including correlation analyses) in other NMD populations were presented in the previous secondary endpoint qualification opinion package (refer to Appendix 2 of the EMA/CHMP/SAWP/178058/2019 package for additional details).	• Results from the generalization to other NMDs provide evidence to support the primary endpoint application.	4.2

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; 6MWT = 6-minute walk test; CGI-C = Clinical Global Impression of Change; CHMP = Committee for Medicinal Products for Human Use; CNM = centronuclear myopathy; COA = clinical outcome assessment; DMD = Duchenne muscular dystrophy; EMA = European Medicines Agency; FSHD = facioscapulohumeral muscular dystrophy; ICC = intra-class correlation; IMP = investigational medicinal product; LGMD = limb girdle muscular dystrophy; MCID = minimal clinically important difference; MCT = meaningful change threshold; MDC = minimal detectable change; NMD = neuromuscular disease; NSAA = North Star Ambulatory Assessment; PODCI = Pediatrics Outcomes Data Collection Instrument; ρ = Spearman correlation coefficient; RMS = root mean square; SD = standard deviation; SEM = standard error of measurement; SMA = spinal muscular atrophy; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

1.5. Remaining Gaps and a Brief Overview of how these will be Addressed

The present qualification package follow-up includes data from different sources that confirms all metric properties of SV95C in DMD including the robustness, construct validity, and sensitivity to change. Nevertheless, this qualification is based on data collected on patients older than 5 years and additional data is required to extend the validity of SV95C to younger ambulant patients with DMD. Indeed, with the walking ability acquisition, growth, and the relatively low impact of the disease in children aged 2 to 5, the evolution of SV95C is not yet established and might present more likely with an improvement and a higher variability.

Long-term data collection will help to define how SV95C change will be predictive of important milestones further down the line, such as loss of running or stair climbing capabilities. Given the number of factors that may interfere, such as incidental fracture or infection, large data collection and long term follow up are required to establish the predictive value of the measure. The qualification as primary endpoint will contribute significantly to this data collection.

We also suggest, as recommended on the guidance "Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy (2015)" published by EMA, to use a relevant secondary endpoint assessing muscle or strength function in the design of the future clinical trials using SV95C as a primary endpoint to confirm consistency.

In addition, while overall data are in favor of a MCT of about 0.1 m/s, the clinical relevance of the change from patients perspectives was determined only on results from questionnaires completed by parents and clinicians during a clinical trial prematurely stopped due to lack of the investigational medicinal product efficacy leading to a high MCT of about 0.2 to 0.3 m/s, as compared with the MDC calculated based on the distribution of 0.127 to 0.194 m/s regarding the level of confidence interval from 80% to 95%. Collecting additional data with patient reported outcome through health-related quality of life questionnaires will help to strengthen the anchoring and refinement of a MCT for the SV95C.

The present qualification package also includes data from similar conditions justifying the qualification of secondary endpoint for other progressive NMDs characterized by proximal muscle weakness. More data including a broader range of disabilities and patients are needed to capture changes in such more slowly progressive diseases to claim primary qualifications in these diseases.

1.6. Conclusion

Based on the totality of evidence presented, we demonstrate that SV95C is an accurate digital and clinically meaningful outcome assessing passively the maximal speed of a patient in a real-life setting through a medical device worn by ambulant patients living with DMD. The evidence supports its use as a primary efficacy endpoint in clinical trials targeting ambulant patients with DMD and as a secondary endpoint in other progressive NMDs characterized by proximal muscle weakness leading to ambulation disorder such as SMA, CNM, or FSHD.

2. Statement of the Need for and Impact of SV95C in Drug Development

2.1. The Intended Application of SV95C in Clinical Drug Development

SV95C is a COA generated from signals passively collected through sensitive sensors located in a wearable device and system[a], such as the CE marked Class I medical device called ActiMyo® (Section 7.4) and analyzed asynchronously. It represents the maximal speed of subject's strides performed in a real-life setting, whereby the 95th percentile is taken as the threshold of maximal speed (i.e., 95% of the strides performed by the subject are slower than SV95C and only 5% of the strides performed are faster).

Measuring disease progression and response to treatment in progressive NMDs characterized by a proximal muscle weakness, such as DMD, is a challenge for all clinical development plans. However, the number of potentially effective therapeutic approaches in DMD are increasing,[5] and thus the demand for validated COAs to demonstrate a clinically meaningful therapeutic response over time in clinical trials (e.g., 1 year) is higher than ever. SV95C has particular advantages over other gold standard COAs (e.g., 6MWD) or other functional scales for this purpose (refer to Section 2.3 for additional details on currently available COAs). As the data is collated continuously over a period of time, and in a real-life, home setting, SV95C represents the maximal speed at which a subject moves around in an uncontrolled environment while a more traditional measure of functional capacity, such as 6MWD, represents the maximal speed at which a subject is able to walk in a controlled environment only during the limited timepoint of taking the test. This also means that SV95C does not rely on patient motivation or fatigue level at the time of the test or subjective assessment. Thus, SV95C offers a more clinically relevant assessment that is less sensitive to different biases of standardized measurements and is more representative of the patient's real motor function than the functional outcomes captured by other measures. Therefore, SV95C is a more appropriate outcome to use in clinical trials that aim to demonstrate the efficacy of a treatment in maintaining, improving, or reducing the decrease of the walk ability of DMD patients, or in natural history studies that aim to characterize the course of the disease.

In DMD patients who are losing ambulation, the maximal stride velocity decreases over time. Any stabilization or an improvement in this would thus be indicative of an improvement in condition or delay of progression. SV95C could therefore be used to assess the change in the stride velocity induced by an investigational medical product by either a comparison between pre- and post-treatment (intra-subject comparison) or a comparison between treated and untreated patients (inter-subject comparison). SV95C may be also used to assess the change from baseline in stride velocity over time during the natural course of the disease, which could be used as part of a broader measurement strategy.

The CHMP has previously qualified SV95C as a secondary endpoint, based upon the information presented in the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019). At the time of the earlier qualification as a secondary endpoint, the CHMP concluded: *"the SV95C measured at the ankle as an appropriate endpoint in studies to support regulatory decision making on medicines for the treatment of Duchenne Muscular Dystrophy (DMD). Based on data provided and State of the art science in the field, CHMP considers that for ambulant Duchenne Muscular Dystrophy (DMD) patients 5 years of age and above, SV95C is an acceptable secondary endpoint in pivotal or exploratory drug therapeutic studies for regulatory purposes when measured by a valid and suitable wearable device, to quantify a patient's ambulation ability directly and reliably in a continuous manner in a home environment and as an indicator of maximal performance. SV95C measured at the ankle may also be used to quantify a patient's baseline performance in such studies."*

However, regarding use of SV95C as a primary endpoint to assess new drug efficacy in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity in real-life of patients with DMD, the CHMP stated that: *"regarding use as primary endpoint for pivotal trials in this setting, although promising, more robust data gained with additional patients and longer follow-up could be beneficial: thus, strengthening the long-term correlation of SV95C with functional tests, expanding normative data and further supporting the justification of the clinical relevance of the proposed MCID in the PEP setting is recommended."*

Therefore, in this follow-up qualification package, additional evidence which builds upon that previously presented in the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019) is presented to address the comments raised by the CHMP at that time, to confirm and further inform the different measurement properties of the SV95C, and to support its use as a primary endpoint to assess new drug efficacy in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity of patients with DMD (refer to Table 3 on page 16 for a brief summary of the results and analyses). This includes evidence of content validity from qualitative and survey based research with clinical experts and patients and their caregivers [refer to Section 3.2.1] and additional quantitative data [test-retest reliability, construct validity, responsiveness, and MCT analyses; refer to Section 3.2.2]. The additional data presented in this qualification opinion package are consistent with the previous data presented in the secondary qualification of the SV95C.

The targeted population to use SV95C as a primary endpoint in clinical trials includes ambulant patients genetically diagnosed with DMD (ambulant meaning able to walk 10 steps [5 strides] independently). The legacy device was used by patients from 5 years of age or older, but no limitation is foreseen for younger patients if they accept to wear the device long enough to get a sufficient amount of data to compute accurate variables. The device is also considered to be suitable for use by patients from any country. With SV95C being a digital COA collected in a real-life setting, there are no foreseen limitations due to language or culture.

2.2. The Disease in Which SV95C will be Applied

As also outlined in the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019 (refer to pages 14 and 15), DMD is a rare and devastating childhood disease, affecting 1 in 5,000 boys.[10] DMD is an X-linked disorder caused by mutations in the *dystrophin* gene. The disease causes progressive muscle weakness, which is frequently identified in infancy and the early toddler years when they present with delayed motor milestones.[11] Children with DMD have pseudohypertrophy in their lower extremities, difficulties bending their knees, which also affects their ability to walk, run, and use stairs unassisted.[12] Symptoms are usually present around 2 to 3 years of age. Difficulties keeping up with peers in physical activities affects boys 4- to 5- years old. Most are wheelchair dependent by 10 to 12 years of age and need assisted ventilation by approximately 20 years of age. Furthermore, most patients will die from cardiac and/or respiratory failure between the ages of 20 and 40.[13]

The earliest challenges within DMD because of progressive muscle weakness include difficulties with climbing stairs, frequent falling, a waddling gate, inability to walk as fast as their peers, and inability to walk long distance. These symptoms can all have an impact on the level of ambulation in patients with DMD. In addition, different natural history studies have illustrated that the walking speed of patients living with DMD, as measured in a controlled environment by the maximal distance walked in 6 minutes, declines with age.[14,15] Furthermore, the decline of the top walking speed has been shown to be predictive of a loss in ambulation, an important milestone in DMD.[3]

Currently, there is no cure for DMD.[13] Therefore, a valuable treatment benefit in DMD patients would

be to at least maintain or delay the loss of muscle function and strength.[13] Glucocorticoids (prednisone and Emflaza® [deflazacort]) are often recommended to be prescribed to patients with DMD. Glucocorticoid treatment allows the patient to maintain muscle strength and pulmonary function for as long as possible. Other therapeutic options include, but are not limited to, small molecules targeting nonsense mutations, gene therapy, stem cell transplant, exon skipping, and utrophin upregulation.[13] Recently, Translarna™ (Ataluren) has been granted conditional marketing approval by the EMA (for nonsense mutations that represent about 10% of the mutations) and Exondys 51™ (Eteplirsen) and Vyondys 53 (Golodirsen) by the Food and Drug Administration (for deletions theoretically treatable by exon skipping 51, 53 and 45 that represent about 15%, 9% and 13% of affected DMD boys respectively).[16]

DMD is the most frequent muscular dystrophy in childhood. Several other NMDs present with similar proximal muscle weakness leading progressive difficulties in ambulation and ultimately in loss of ambulation. These conditions are much rarer, and the clinical development that are ongoing or are planned are facing with the same obstacle as DMD. As they share the same feature of proximal muscle weakness and as they are much rarer to allow such a validation process, we propose to include them in the present application by providing data in several of these conditions. The rarity of these diseases should indeed not justify less efficient clinical developments and rather promotes the use of the most sensitive measures to ensure these patients prompt access to treatments that can demonstrated efficacy on limited cohort- which implies sensitive measures. In this context, we propose the use of SV95C as COA and secondary endpoint also in: Limb girdle muscular dystrophies, Becker muscular dystrophy (BMD), SMA Type 3, CNM, FSHD, Pompe disease, or any progressive muscular disease with a clear involvement of the lower girdle. This group does not include diseases with a more distal phenotype and late proximal involvement such as myotonic dystrophy (MD) Type 1, or distal myopathies.

2.3. Currently Available Tools in Patient Care and Clinical Drug Development

The number of potentially effective therapeutic approaches in DMD are increasing,[5] and thus, the demand for validated COA measures to demonstrate a clinically meaningful therapeutic response over time in clinical trials (e.g., 1 year) is higher than ever. The primary endpoints most often used to demonstrate a treatment effect in clinical trials targeting patients with DMD are functional measures that are based on performance on tests that are conducted in a clinic setting, such as change in the NSAA[4] score or change in the maximal distance walked in 6 minutes (6MWD).

The 6MWT is used to capture the distance walked by a patient in 6 minutes (6MWD) at the pace of maximal effort (running is not allowed), on a flat, hard surface, turning between two 25 m-distanced cones. 6MWD has been used as the primary endpoint evaluation in several pivotal clinical trials, and to support 2 Investigational New Drug Application filings in the United States (US) and in the European Union (NCT02255552 and NCT03179631, respectively), and is thus considered to be a gold-standard.[2,17-19] To perform the 6MWT appropriately, adequate training of evaluators is mandatory, and a standardized script is used by all evaluators. However, there remains an element of subjectivity, for instance, in the way the evaluators encourage the patients through the test.[3,20] It is also heavily dependent upon the patients' motivation and clinical condition at the precise time of assessment.

The NSAA is a clinician reported rating scale that includes the evaluation of 17 different functional activities, including a 10-m walk/run, rising from a sit to standing, standing on 1 leg, climbing a box step, descending a box step, rising from lying to sitting, rising from the floor and jumping. Patients are graded by clinicians (who are trained on the use of the NSAA) on a 3-point scale[21] (where 0 = "unable to achieve independently" 1 = "Modified method but achieves goal independent of physical assistance from another person," and 2 = "normal achieves goal independent of physical assistance from another person"; the total score ranges from 0 to 34 (NCT03039686, NCT03439670, NCT03354039,

NCT03907072).[21] It is a subjective measurement, and the clinically meaningful change is very high (8 to 9 points on the linear scale).[22-24]

The 4SC[2] is also used as a primary endpoint, though less commonly. This is the minimal time required by the patient to climb 4 stairs. It not only implies power, but also motor praxis. This timed test may be very rapid, in the order of 2 seconds, which overpowers the reflex time of the patients and the physiotherapist (e.g., NCT02851797, NCT02310763, NCT01254019, NCT01954940).[25] This leads to either an increase of the number of patients per trial, or to an increase of trial duration to allow for this level of noise in the data.

These primary endpoints, assessing the change in the top performance during functional measures based on performance on tests conducted in a clinic setting, are supported by secondary endpoints that include other functional assessments (such as the MFM20 or MFM32 or measures of lower limb strength), patient-reported outcome measures (such as the Pediatric Outcome Data Collection Instrument [POD-CI], Pediatric Quality of Life Neuromuscular Module [PedQoL-NM] or measures of activities of daily living, and biological endpoints and more invasive assessments (such as muscle biopsy or magnetic resonance imagery to assess the pathophysiology).

Akin to the primary endpoints outlined above, the MFM is a similar functional test commonly used in clinical practice that assesses the severity and progression of motor function in patients with an NMD. Items are rated on a 4-point scale (ranging from 0 "cannot perform the task, or cannot maintain the starting position" to 3 "performs that task fully and 'normally'; the movement is controlled, mastered, directed, and performed at constant speed") that assess 3 areas of function (standing position and transfers, axial and proximal motor function, and distal motor function).[6,7]

Alternatively, the POD-CI, PedQoL-NM and activities of daily living measures are typically based upon self or parent report of functional abilities within the real-life setting. Such measures are designed to capture the broader patient experience beyond the clinic setting. Although such measures have been shown to be related to the more objective measures such as the 6MWD, be sensitive to the changes in functional status over time in DMD,[26] and are useful to demonstrate the impact of the condition upon the patients' lives as part of a comprehensive COA measurement strategy, they are based upon subjective reports of activities recalled over a certain period of time and thus represent typical level of functioning and not maximal functional capacity as with SV95C.

The traditional functional tests currently used as primary endpoints, however, have major limitations. All these validated COA measures of functional performance are conducted in a controlled environment during a clinical assessment. To use those existing assessments in a multiple center clinical trial, a standardized training for evaluators is mandatory to minimize bias related to evaluator.[27] In addition, all of these assessments are episodic and provide only a glimpse of what is assumed to be the patient's maximal functional ability, based upon an assessment taken in a clinic setting.[8] Data collected continuously over a much longer period of time and in a natural setting, whether at home, school or work, would be far more reliable, objective, and accurate than several 6MWTs, taken in a hospital or clinic setting, weeks or even months apart. These concerns were reflected by comments received during a public consultation of SV95C as a secondary endpoint in DMD (EMA/532515/2018) from the Duchenne Community Advisory Board (CAB) during the public consultation on the previous SV95C EMA qualification process for use as a secondary endpoint.

Additionally, timed tests are mostly peak performance tests where patients are asked to performed tasks as fast as they can and results might be affected by multiple factors such as a patient's age, motivation, compliance, mood, time of day, training at home before the test, or fatigue from travelling to the hospital, especially in the context of a rare disease when the patient often does not live close the investigation center. This induced variability around existing COAs requires a high number of

patients enrolled in clinical trials to reach statistical power (for example NCT00592553: 174 patients; NCT01826487: 230 patients; NCT02500381: 222 patients; and NCT01865084: 331 patients; all of which include the 6MWD as primary; NCT03039686: 159 patients, which includes the NSAA as primary; and NCT02851797: 213 patients, which includes the 4SC as primary) and an increase in study duration to 18 (NCT02851797) or 24 months (NCT02500381) as no change has been typically observed with less than 1 year of follow up. In contrast, good stability with low variability (4.41%) was reported for the 95th percentile of stride velocity based on 180 hours of wearable device and system use in 28 patients (refer to the previous secondary endpoint qualification opinion package [EMA/CHMP/SAWP/178058/2019; pages 27 to 32]). These timed tests are also very demanding for patients suffering with a muscle weakness, and in comments received during a public consultation of SV95C as a secondary endpoint in DMD (EMA/532515/2018), The Duchenne Parent Project – Belgium stated, "*The 6MWT is really cruel and NSAA when captured occasionally at the hospital is not enough reliable given the little number of boys in DMD CT's.*"

Furthermore, the medical community agrees that there is a need for better and more meaningful endpoints than those currently used to assess efficacy of new therapies.[8] In the comments obtained during public consultation of SV95C as a secondary endpoint in DMD measured by a valid and suitable wearable device (EMA/532515/2018)[e], Prof. Erik Niks, child neurologist from the European Academy of Neurology indicated that "*In this disease, the field is facing many ongoing and planned clinical trials, and there is a need for better endpoints than the ones currently used.*"

SV95C addresses the issues with existing COAs described above and presents a significant advantage over the classic 6MWD or clinical scales. It is a digital COA based on a wearable device and system that passively collects data. Measuring the motor function of patients in a real-life setting with accurate and sensitive sensors such as SV95C offers a more clinically relevant assessment that is less sensitive to different biases of in-clinic assessments. Evaluating the patients' motor function in a real-life setting through continuous home monitoring allows a more granular and objective assessment of daily motor activity, which is not influenced by motivation and clinical condition during an acute episodic assessment. Thus, it considerably decreases the variability of assessment, which would allow for a smaller number of patients to be included in a study. It is thus more representative of the patient's real motor function.

Consideration should also be given to the impact that attending assessments at regular intervals has on patients and their families, who are required to adapt their life around those visits. Among other factors, these visits may contribute to a loss in work time/productivity for caregivers and school days lost.[28,29] The importance of this aspect has been reinforced with the recent COVID pandemic during which home monitoring offers a safe and viable alternative to site visits which have been globally suspended in many locations. Clinical trials that rely on the collection of a primary outcome were compromised by preventing travel and access to investigational sites and had to be put on hold or adapt their designs.[30] Using a wearable device enables remote data collection to allow the completion of trials even when in-clinic visit are not possible. It is likely to also overcome variations in practice encountered across different centers/countries, which also has a significant impact on global studies. This considerably decreases the variability of assessment, which would allow for a smaller number of patients to be included in a study. Assessment of motor function in daily life therefore offers a much more clinically relevant and powerful outcome measure to demonstrate efficacy in DMD clinical trials. Using SV95C will therefore address the issues with existing COAs and serve to measure the change in the top performance by assessing the maximal speed gait.

As with the more traditional functional assessments, SV95C is proposed as a primary endpoint within a

[e] Overview of comments on 'Stride velocity 95th centile as a secondary endpoint in Duchenne Muscular Dystrophy measured by a valid and suitable wearable device' (EMA/532515/2018)

broader measurement strategy, supported by secondary endpoints capturing other aspects of mobility and functional impairment in addition to patient centered outcomes evaluating the broader impact of these limitations.

2.4. Characteristics of SV95C

SV95C is a clinical outcome measure derived from a device that is passively assessing the maximal walking speed of ambulant patients living with DMD, in a real-life home setting. It represents the maximal speed of subject's strides performed in a real-life setting, i.e., 95% of the strides performed by the subject are slower than the SV95C and only 5% of the strides performed are faster. The difference between SV95C and existing COAs such as the 6MWD, which also measures walking speed, is that SV95C captures this in an uncontrolled environment whereas the 6MWD is assessed in a controlled setting.

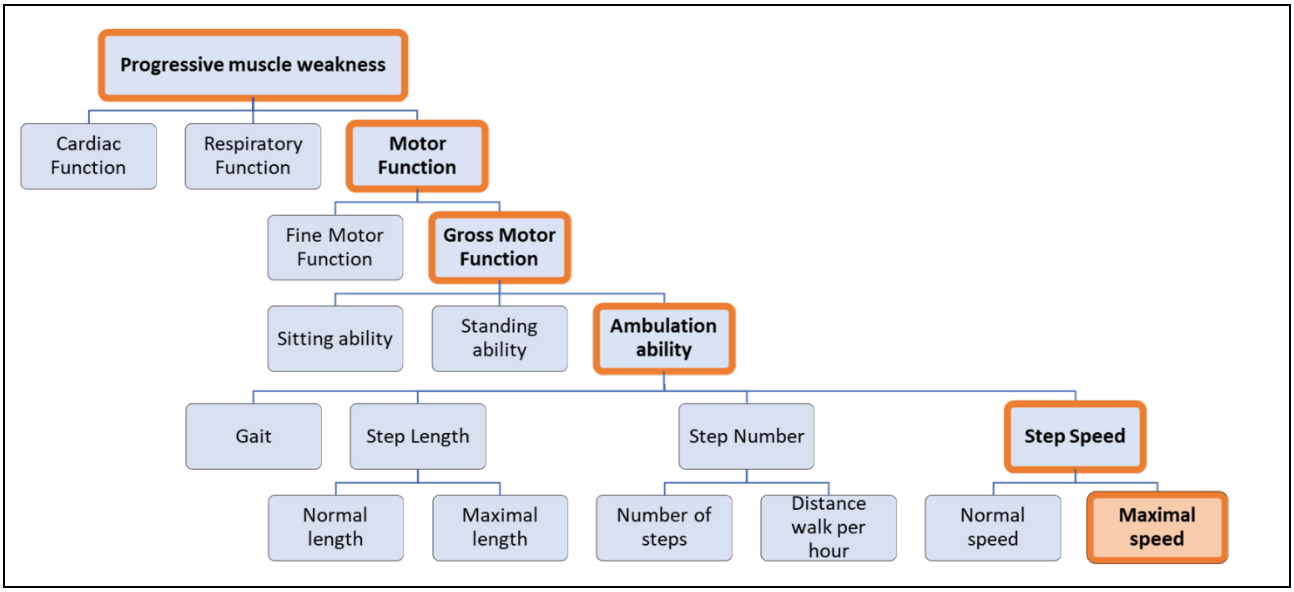
SV95C is computed from signals passively collected through highly sensitive sensors located in a wearable medical device and system developed based on magneto-inertial technology (CE-marked ActiMyo®) worn at the ankle of DMD patients. The patient (or his/her caregiver) fits the device every morning and puts them back on a docking station at night. Data are passively collected each time an equipped patient is moving. The user is not required to interact with the system (e.g., start button, log-in, or other electronic system) beyond unplugging and wearing the device. Data are stored in an internal memory inside each watch-like device and transferred to the docking station, every night, when they are put to charge. The strides trajectories are reconstructed from the data provided by the wearable device attached to the ankle. The integration of all collected data on a defined period of time allows to calculate different walk parameters (stride length, stride speed, walked distance).

The ActiMyo® system is composed of 3 parts fulfilling different sequential functions:

1) The data acquisition system, which is itself subdivided into 2 parts:
    - Two recording wearable sensors worn by the patient, including accelerometers and gyrometers, calibrated after production and regularly throughout the lifetime of the device.
    - A docking station, located in the patient's home, that retrieves data from the sensors, recharges the batteries, and transmits the data to the software platform.
2) The software platform for data storage and monitoring by a project manager.
3) Analysis software for computing statistical variables on the recordings by a trained analyst.

The conceptual framework of the maximal speed, characterized by the SV95C, is based on the decline of ambulation abilities of patients living with NMDs due to progressive proximal muscle weakness, which leads to loss of motor function. The SV95C relates specifically to gross motor function and the impact on ambulation ability. The test picks upon the maximal speed of the steps taken. This is illustrated in Figure 1.

Figure 1: SV95C Conceptual Framework



In the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019, it was demonstrated that the SV95C when measured with the wearable ActiMyo® device is accurate, reliable, sensitive to change, and clinically relevant based on the correlations to existing COAs (6MWD, NSAA, and 4SC test). Furthermore, the device and algorithm remain unchanged from what was outlined in the prior application, and therefore still meets all required regulatory standards for digital medical devices (cf. Certificate of conformity, Section 7.4). All data are pseudonymized (patient ID). When the device is not connected to the Internet, data are stored in a USB drive entirely encrypted. During automatic upload, data are encrypted through SSH. At Sysnav premises, data are stored and analyzed in a dedicated and secured server.

3. Methodology and Results (DMD Population)

3.1. Methods

3.1.1. Qualitative Evidence (Content Validity)

Further information to consolidate evidence of the clinical relevance of the SV95C is presented in the current application, based upon data from healthcare professionals (HCPs), pharmaceutical companies, and patient communities. This information was collected from the following sources:

- Qualitative data and insights designed and collected in partnership with patient organization in Europe and US, from an online survey with 549 responders who live with or assist patients living with NMDs. Patient associations who participated and recruited for the survey cover DMD and other NMDs including LGMD, MD, FSHD, SMA, and centronuclear and myotubular myopathy (CNM/MTM; see Section 7.2). Of the 549 responders, 92 were living with, or caring for someone with, DMD (14 patients and 78 caregivers).
- Opinions and feedback from 52 worldwide HCPs (physiotherapists [57%], study coordinators, study nurses or study managers [34%], Physician [9%]) collected through an online survey on experiences with ActiMyo® directed to site staff trained to use ActiMyo®; and 8 solicited letters of support from neurologists, child neurologists, and physiotherapists from Europe (Belgium, France, Hungary, Poland, Romania), the United Kingdom, and the US (refer to Sections 7.1 and 7.3 for a copy of the online survey and letters of support).
- Comments provided by nonprofit organizations, industries, and the broad scientific community during the public consultation of the previous SV95C EMA qualification process for use as a secondary endpoint.
- Responses from HCPs, patients and caregivers outlined within the Parent Project Muscular Dystrophy (PPMD) 2018 annual congress report[31] and letters of support from the patient association.

The HCP survey included questions to explore use of ActiMyo®, experience of training for use of the device, to elicit HCP reports of feedback they have received from patients using the device and any difficulties they have experienced, and opinions on the potential uses of ActiMyo® and the variables it captures. The survey was distributed to site staff who had been trained to use ActiMyo® as part of clinical trials and other research. Data was collected between 11 and 25 June 2020.

The patient/caregiver survey was designed to determine the patient relevance of passively measuring the maximal ambulation speed in an uncontrolled environment when assessing the efficacy of a new drug. The online survey was developed in collaboration with NMD experts (a child neurologist and a physiotherapist) and American patient organizations (PPMD and Myotubular Trust). It was distributed internationally via a number of US, United Kingdom, Belgian, French and global patient organizations between October 2020 and January 2021. The survey objectives were the following:

- To collect what was important to patients/caregivers in terms of ambulation.
- To determine what were their first symptoms, how the disease impacts their mobility and their family activities, which functions they would like to see maintained, improved, or restored by a treatment, what they consider as a clinical change in terms of ambulation improvement, and if they would accept wearing a wearable device at home to monitor their walking abilities.

Although not involved in the design and conduct of the survey, survey results were analyzed by Clinical Outcome Solutions, a company specialized in measuring and understanding Patient Reported Outcomes (PROs), Clinician Reported Outcomes (CROs), Observer Outcomes (Parent, Teacher, or

Guardian), and Screening Tools in the context of specific clinical conditions. A copy of a report of the survey, which includes the survey questions, is provided in Section 7.2. The report does not contain results for all questions of the survey, e.g., questions regarding upper limb motor function were not analyzed.

The PPMD report is a summary of the results of the live polling that was conducted throughout the PPMD annual conference, which could be completed by all who joined the conference in person and at home. According to the report, the respondents were caregivers (50%), industry professionals (16%), and HCPs (11%). Most were from across the US, though 11% were international.

3.1.2. Quantitative Evidence

3.1.2.1. Population

The intrinsic properties of SV95C (i.e., accuracy and reliability) have been demonstrated in the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019 (refer to pages 5 and 6).[32] In the present dossier, the clinical properties of the SV95C using data collected from the 45 European DMD patients from the previous qualification opinion dossier were supplemented with 80 additional American, European, and Australian patients living with DMD (125 patients total) along with 66 healthy aged-matched control subjects without any muscular condition. These additional patients were mainly enrolled in a European natural history study (NHS; ActiLiège-Next – 2019/343), clinical trials in which the ActiMyo® device was used as a secondary outcome measure (NCT03039686 and NCT03907072) or were in-clinic patients starting corticosteroids. (Refer to Table 1 for the sample size for each study, Table 4 for the population characteristics, ActiMyo® configuration, and recording periods used in each clinical study, and to confidential appendix Section 7.8 for the study details).

Table 4: Origin of Data Used in the Opinion Follow-up Dossier

Study Reference Number	Pathology	Selection Criteria	N	ActiMyo® Configuration	Recording Periods
NHS-A	Healthy subjects	Without any muscle condition	62	Ankle / Wrist	RP = 30 days 2 RP 1 year apart
	DMD	Starting corticosteroids	2	Ankle / Wrist	Continuous recording up to 13 months
NHS-B	Healthy subjects	Without any muscle condition	4	Ankle / Ankle	RP = 30 days 2 RP 1 year apart
	DMD	Stable in use of corticosteroids	11	Ankle / Ankle	Continuous recording
	DMD	Starting corticosteroids	2	Ankle / Ankle	Continuous recording
NHS-C	DMD	Stable in the use of corticosteroids	11	Ankle / Wrist	Continuous recording up to 9 months
CT-A	DMD	Deletion in Dystrophin gene treatable by exon 53 skipping	34	Ankle / Wrist	Continuous recording up to 20 months

Study Reference Number	Pathology	Selection Criteria	N	ActiMyo® Configuration	Recording Periods
		Stable in the use of corticosteroids			
CT-B	DMD	Stable in the use of corticosteroids	51	Ankle / Ankle	RP = 45 days Up to 5 RP, every 3 months
CT-C	DMD	Deletion in Dystrophin gene treatable by exon 51 skipping Stable in the use of corticosteroids	7	Ankle / Wrist	RP = 30 days 1 RP at baseline
In clinic patients	DMD	Starting corticosteroids	7	Ankle / Wrist	Continuous recording up to 24 months

DMD = Duchenne muscular dystrophy; RP = recording period

N = number of subjects equipped with ActiMyo®. In the ankle/wrist configuration, participants were asked to wear the sensors on their dominant side. In the ankle/ankle configuration, results from the sensor located on the dominant side were used.

Of note, data was pooled from each study to provide a sufficient sample size for the analyses described in the following sections. However, based on the availability of data for each participant, not all 125 DMD patients and 66 healthy controls were able to be included for each analysis; the N therefore may differ per analysis and is reported within the results section. Patients are listed in appendix Section 7.9.

3.1.2.2. Test-retest Reliability

Test-retest reliability consists of measuring the degree to which a device measures the outcome the same way at 2 points in time, under the same assessment conditions. Test-retest reliability was assessed by calculating an intra-class correlation coefficient (ICC) in patients with DMD with measures performed 1 month apart in 2 successive recording periods. Specifically, a 2-way random effect model was employed to calculate absolute agreement for the average measures in which the first month recording period were compared to the second month for 52 patients who recorded at least 50 hours on each subperiods. Results were further supported by Bland-Altman plots.[33-35]

The patients whose data were used in this analysis are listed in Table 5.

Table 5: List of DMD Subjects With at Least 50 Hours of Recordings at Months 1 and 2 used in the Assessment of Test-retest Reliability

Timepoint	Patient ID
M1 + M2	1, 2, 3, 4, 5, 6, 7, 8, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 81, 83, 84, 85, 86, 87, 88, 89, 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114

DMD = Duchenne muscular dystrophy; M1 = Month 1, M2 = Month 2

3.1.2.3. Construct Validity

The construct validity is the degree to which a measure assesses what it is intended to measure.

3.1.2.3.1. Known-groups Validity

Known groups validity is a form of construct validity and is the ability of a measure to discriminate between groups of individuals known to differ in terms of the construct of relevance, i.e., between clinically distinct groups hypothesized *a priori*.[36] To confirm the clinical validity of the SV95C demonstrated in the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019, 125 patients with DMD (45 patients from the previous qualification opinion dossier and 80 additional patients) between the ages of 5 and 14 years were compared with 66 control subjects (37 subjects from the previous qualification opinion dossier and 29 additional subjects) 6 to 14 years of age without any muscle condition. In addition, comparisons were performed in patients and healthy controls stratified by age groups (5 to 7 years of age in the DMD population and 6 to 7 years of age in the healthy control population versus 8 to 14 years of age in both populations) given that performance is expected to deteriorate with age. Comparisons were performed with independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-Wallis Test (when more than 2 samples). The patients and healthy controls whose data were used in this analysis are listed in Table 6.

Table 6: List of DMD and CTRL Subjects Involved in the Known-groups Validity Analysis

Timepoint	Patient ID
Baseline	1, 2, 3, 4, 5, 6, 7, 8, 9, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 117, 118, 119, 120, 121, 123, 124, 125, 126, 127, 129, 130, 131, 132, 133, 134, 135, 138, 140, 145, 146, 147, 148, 149, 154, 156, 158, 159, 160, 161, 162, 163, 164, 165, 168, 169, 170, 171, 172, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 187, 188, 189, 190, 191, 193, 195, 196, 198, 200, 201, 202, 203, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 220, 221, 222, 223, 224, 230, 231, 234, 235

CTRL = Control population, i.e., subjects without any muscle conditions; DMD = Duchenne muscular dystrophy

3.1.2.3.2. Convergent Validity

Convergent (or concurrent) validity is the degree to which the score on a measure is associated with scores on other COA tools that measure the same construct. The convergent validity of the SV95C was assessed by cross-correlating SV95C with existing COA measures (6MWD, NSAA, and 4SC) using data from 107 patients in total (including the 45 patients from the previous secondary endpoint qualification opinion package [EMA/CHMP/SAWP/178058/2019], and 62 additional DMD patients with available data). Both parametric and non-parametric correlations were used. Correlations including longitudinal correlations between SV95C and the existing COAs were also computed on all available data after 3, 6, 9, and 12 months of follow-up. The patients whose data were used in these analyses are listed in Table 7.

Table 7: List of DMD Subjects with SV95C, 6MWD, NSAA, 4SC Available at Baseline and at

Each Timepoint used in Assessment of Convergent Validity

Timepoint	Patient ID
Baseline	1, 2, 3, 4, 5, 6, 7, 8, 9, 15, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 118, 119
3 months FU	31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 51, 52, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 66, 67, 68, 69, 70, 71, 72, 73, 75, 77, 78, 79, 80
6 months FU	33, 34, 37, 38, 40, 43, 46, 48, 49, 51, 52, 54, 56, 58, 60, 68, 69, 72, 74, 75
9 months FU	31, 33, 34, 37, 40, 44, 45, 46, 47, 51, 52, 54, 55, 57, 60, 64, 67, 68, 69, 71, 74, 75, 79, 80
12 months FU	31, 33, 34, 38, 44, 45, 46, 47, 51, 55, 68, 69, 72, 75, 80

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy; FU = Follow up; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

3.1.2.4. Responsiveness (Ability to Detect Change)

Responsiveness (or sensitivity to change) refers to the ability of an assessment to detect change where change exists. The sensitivity to change of the SV95C was assessed by studying the natural change over time at 6 and 12 months of the SV95C measured in a group of patients with DMD. The sample size was increased respectively by 22 patients and 15 patients compared with the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019) to make a total of 53 patients at 6 months and 27 patients at 12 months. The changes of SV95C at 3 and 9 months were also assessed in 81 and 37 DMD patients, respectively. In addition, the natural course of the disease was followed over the course of 12 months in a total of 17 patients. The same analyses were also performed with patients stratified by age group. Treatment effect through the initiation of corticosteroids was also assessed in 11 DMD patients (11 patients at Baseline and Months 3 and 9, 7 patients at Months 6, and 5 patients at Month 12). The patients whose data were used in these analyses are listed in Table 8.

Table 8: List of DMD Subjects used in the Assessment of Responsiveness

Timepoint	Patient ID	
	Natural Course of the Disease	Treatment Effect
3 months FU	1, 2, 4, 5, 15, 19, 20, 23, 28, 29, 31**, 33**, **34**, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, **46**, 47, 48, 49, **51**, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 66, 67, **68**, **69**, 70, 71, 72, 73, 74, **75**, 76, 77, 78, 79, 80, **82**, **83**, 84, 85, 88, 89, 92, 93, 95, **96**, **97**, **98**, **99**, **100**, 101, 102, 103, 105, 106, 108, **109**, **110**, **111**, 112, 114	115, 117, 118, 120, 121, 123, 124, 125, 126, 127, 133
6 months FU	19, 20, 23, 25, 28, 29, 33, 34, 35, 37, 38, 40, 41, 42, 43, 46, 48, 49, 51, 52, 53, 54, 56, 58, 60, 61, 63, 65, 68, 69, 72, 74, 75, 81, 82, 83, 84, 85, 87, 89, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, 103, 105, 106, 108, 109, 110, 111, 112, 114	115, 120, 121, 124, 125, 126, 127
9 months FU	31, 33, 34, 36, 37, 40, 44, 45, 46, 47, 49, 51, 52, 53, 54, 55, 57, 60, 64, 67, 68, 69, 71, 74, 75, 76, 79, 80, 81, 82, 83, 96, 97, 98, 99, 100, 109, 110, 111	115, 120, 121, 124, 125, 126, 127
12 months FU	31, 33, 34, 36, 38, 44, 45, 46, 47, 51, 55, 68, 69, 72, 75, 80, 81, 82, 83, 95, 96, 97, 98, 99, 100, 109, 110, 111	115, 120, 121, 123, 124

DMD = Duchenne muscular dystrophy, FU Follow up

The 17 patients followed over 12 months are marked in bold

Changes were assessed with a one-sample Wilcoxon signed rank test with null hypothesis being a median change of zero. The standardized response mean (SRM) was calculated in case of significative change as the |mean| divided by the standard deviation (SD). Sample size was estimated based on the following equation: $n = 2*\Phi^2/SRM^2$ with Φ= 2.802; 3.242; 3.605 for α = 5% - β = 20%; α = 5% - β = 10%; and α = 5% - β = 5%, respectively.

3.1.2.5. Meaningful Change Thresholds

3.1.2.5.1. Distribution-based Threshold

The standard error of measurement (SEM) is the amount of change that can be attributed to a measurement error. The SEM was calculated by the formula SEM = SD*SQR(1-ICC) wherein the ICC was calculated based on the specifications provided in Section 3.1.2.2 with a 2-way random effect model employed to calculate absolute agreement for the average measures in which the first 15 days in a month recording period were compared to the last 15 days in the month for 103 patients who recorded at least 50 hours in each subperiod (Table 9).

Table 9: List of DMD Patients with 50 Hours of Recordings During the First 15 Days and the Last 15 Days of the First Recording Period

Timepoint	Patient ID
Baseline	1, 2, 3, 4, 5, 6, 7, 8, 9, 15, 16, 19, 20, 21, 22, 25, 29, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 81, 82, 83, 84, 85, 87, 88, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 110, 112, 113, 114, 115, 116, 117, 118, 119, 120, 123, 126, 127, 129, 130, 131, 132, 133

DMD = Duchenne muscular dystrophy

The minimal detectable change (MDC) is the smallest amount of change that can be detected by a measurement that corresponds to a noticeable change in ability (i.e., the minimum change that is statistically greater than measurement error). The MDC was statistically measured based on the SEM at 95%, 90%, and 80% confidence levels, as well as by considering 0.2 SD, 0.5 SD, and 0.8 SD of the measurement.

3.1.2.5.2. Anchor-based Within Patient Threshold

The within-patient MCT was assessed through an anchor-based approach based on the Pediatrics Outcomes Data Collection Instrument (PODCI) and Clinical Global Impression of Change (CGI-C) scales in 15 and 12 patients with DMD at 48 weeks, respectively (Table 10).

Table 10: List of DMD Patients with PODCI and CGI-C Evaluation After 48 Weeks of Follow-up

Timepoint	Patient ID
CGI-C	31, 33, 38, 44, 45, 46, 47, 51, 55, 68, 69, 80
PODCI	31, 33, 34, 38, 44, 45, 46, 47, 51, 55, 68, 69, 72, 75, 80

CGI-C = Clinical Global Impression of Change; DMD = Duchenne muscular dystrophy; PODCI = Pediatrics Outcomes Data Collection Instrument

The CGI-C is a single item that clinicians completed at the end of the double-blind phase (Week 48 of the study) to rate the change in the patient's global impression of change in DMD from the start of each phase (i.e., from Baseline for the double-blind phase). There are 7 response options: "very much improved," "much improved," "minimally improved," "no change," "minimally worse," "much worse," and "very much worse" coded from 1 to 7. The responses were collapsed into categories indicating any improvement, no change, and any worsening in the analysis of meaningful change. The mean SV95C change during the same period of time was calculated in each change category.

The PODCI is a measure of physical functioning and health-related quality of life outcomes in musculoskeletal conditions, designed for use in either an adult or a pediatric population. It is completed by parents or can be self-reported in an adolescent population. The PODCI consists of 83 to 86 questions with 5 core scales (upper extremity and physical function, transfer and basic mobility, sports and physical functioning, pain/comfort, and happiness) and 1 global functioning scale. Scores for each PODCI subscale range from 0 to 100, with lower scores indicating lower health-related quality of life. The transfers and basic mobility subscore was used from the PODCI as an anchor of change as this was a secondary endpoint in the clinical trial.[17,37] A threshold of 10% in score was used to indicate change, 2010 J Child Neurol).[38] The mean SV95C change during the same period of time was

calculated in each change category (refer to Section 7.5 for a copy of PODCI and CGI-C questionnaires).

The within patient MCT was also assessed through an anchor-based approach using the change over 48 weeks of reference functional tests performed in the CT-B study, namely 6MWD (N=13) and NSAA (N=12) (refer to Table 7) based on their MDC80% (*i.e.* 2.78 points for NSAA and 36.3 m for 6MWD).

3.2. Results

3.2.1. Qualitative Evidence (Content Validity)

Evidence is presented demonstrating the importance of ambulation for patients living with DMD from various perspectives, not only patients and caregivers themselves but also patient advocacy groups, clinical experts, and industry. As part of the initial development of ActiMyo® and selection of SV95C, representatives from each of these bodies were consulted to confirm that stride velocity is a relevant and meaningful endpoint in DMD. Expert panels were held with clinicians, physiotherapists, and patient advocacy groups in the DMD field, and feedback was obtained from initial users of the device in industry (BMS, Roche, WaveLife Science, Solid Bioscience, Genethon). Evidence within the literature also confirmed the need for a measure of mobility that better captured real-life functioning.

Different ways to characterize ambulation were explored through gait parameters or distance walked. In addition, several conceptual variables related to ambulation (stride length, stride speed, distance walked, etc.) were developed. Based on discussions with clinician experts and as reported in the April 2016 project report from the European project name SKIP-NMD, the focus was on variables related to the steps, namely stride length and stride speed. The scientific group lead by Prof. Muntoni concluded: "Briefly, it appears that parameters associated to movement quality and description are much less dependent upon social and environmental parameters than the quantification of movements. The analysis of data collected so far is encouraging and indicates that the variability of the studied variables decrease with averaging time, and is about 1-2% on a 2 week period. This potentially favorably compares with the variability observed in other outcome measures including the 6MWT."

Those variables have then been tested on data collected from patients enrolled in clinical trials where the output from ActiMyo® were used as an exploratory outcome measure. Lastly, because more sensitive to change, the maximal speed at which a patient is able to move around in a real-life setting has been selected by clinician experts as a clinically meaningful outcome.

3.2.1.1. Opinion of HCPs

Experts Recognize the Need of New Endpoints in the NMD Field

In the comments obtained during public consultation of SV95C as a secondary endpoint in DMD measured by a valid and suitable wearable device (EMA/532515/2018)[f], Erik Nilks, pediatric neurologist from the European Academy of Neurology indicated that, *"In this disease, the field is facing many ongoing and planned clinical trials, and there is a need for better endpoints than the ones currently used."* (page 13).

In addition, comments during the public consultation demonstrated that most of the clinicians agreed that the existing COAs (6MWD, 4SC, MFM, and NSAA) often used as primary endpoint to demonstrate a treatment effect in clinical trials targeting ambulant patients with DMD, are highly sensitive to the patient's state on the day of the evaluation (motivation, fatigue, concentration, and well-being) as well as growth and intellectual maturation in children. In addition, the Duchenne CAB, Action Duchenne and the World Duchenne Organization – UPPMD all agreed that wearable devices offer an advantage over existing COAs that are performed in a controlled environment with standardized procedures, requiring the patients and their families to travel to the hospital. In their comments, these organizations confirmed concerns that existing functional tests such as the 6MWT are time consuming and often induce fatigue in the patient. Similarly, such assessments are also time consuming for HCPs, who reported in the 2018 PPMD Annual Conference report,[31] that the biggest barriers to conducting clinical trials were the staff time/capacity (indicated by 30% of the 57 healthcare respondents) and the insufficient time for both clinic and research responsibilities (indicated by 23%). Interestingly, 7% indicated that lack of physical space for the provision of both care and trial assessments was a barrier.

Lastly, in the 2018 PPMD Annual Conference report, the members of the audience from industry (n = 38) considered that the largest barriers to conducting clinical trials in DMD were participant recruitment (indicated by 21%), followed by challenges in endpoint selection (indicated by 18%).

Experts Showed Interest in Digital Endpoints Collected in the Real-life Setting from a Wearable Device

Based upon the letters of support received (see Section 7.3), most HCPs recognize that assessments performed in a controlled environment, mainly for children, did not always accurately reflect the patient for the reasons explained in previous section, but also because children are influenced by the familiarity of the environment. Lena Szabo, Head of Neurology ward in the Second Department of Pediatrics in Hungary stated in their letter of support that the SV95C represents the spontaneous maximal velocity of patients at home, and thus, provides better representation of a patients' real-world top performances. In addition, Laurent Servais, Professor of Pediatric Neuromuscular Diseases in Oxford indicated in their letter of support that the COVID-19 pandemic experience has disrupted the course of several clinical trials and has illustrated the need for collecting data in a real-world setting.

These opinions are consistent with many comments received during the public consultation of SV95C as a secondary endpoint in DMD (EMA/532515/2018) who recognized that a valid and reliable wearable device has advantage over existing COA measures in these respects. Representatives from the Critical Path for Parkinson's, Duchenne Regulatory Science Consortium, Critical Path for Alzheimer's Disease, Patient Reported Outcome Consortium, and the Quantitative Medicine Group on behalf of the Critical Path Institute, Ltd. (CPath) reported that a digital endpoint like SV95C may reduce travel burden to individuals participating in clinical trials. The Duchenne CAB also supported the use of wearable devices because it is more patient relevant than and possibly superior to the 6MWT, outlining

[f] Overview of comments on 'Stride velocity 95th centile as a secondary endpoint in Duchenne Muscular Dystrophy measured by a valid and suitable wearable device' (EMA/532515/2018)

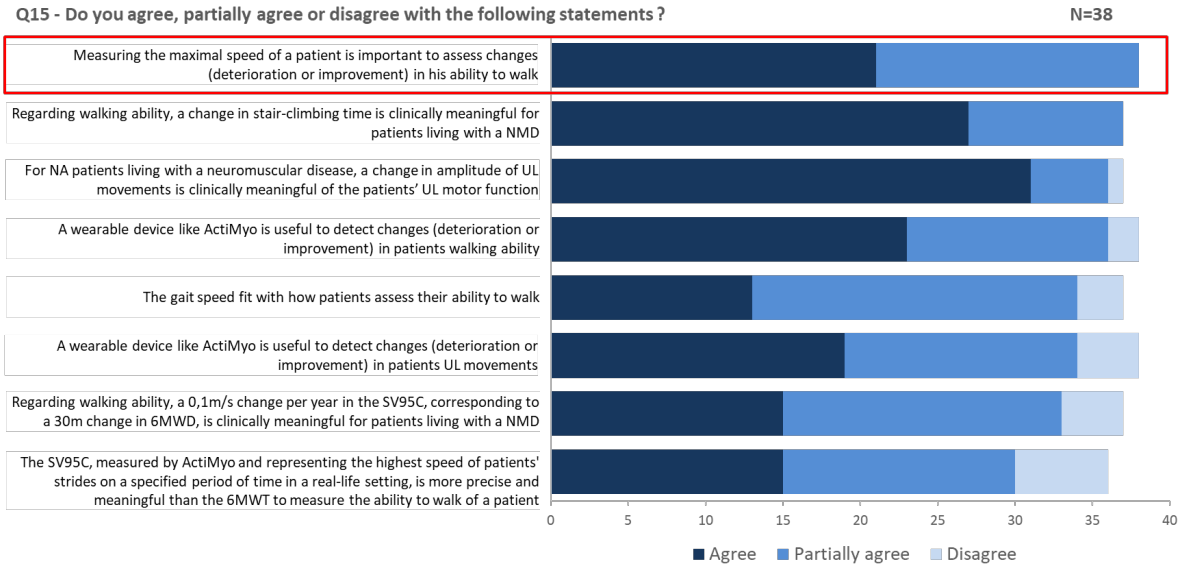
the logistical challenges, subjectivity and bias associated with a clinic-based functional assessment.[39]

Experts Recognize of the Importance of Measuring the Maximal Speed at Which a Patient Walks

The primary symptom of DMD is the gradual loss of top-level performance related to ambulation (such as climbing stairs, running, jumping, and fast walking), progressively leading to the eventual loss of ambulation, which is a very important milestone for patients and caregivers. As declared by Dr Aurore Daron, Child Neurologist in the Belgian Reference Center Neuromuscular Disease in Liege, young children report their inability to walk fast or to run as one of the first complaints, which translates into a decreased ability to socially interact with their peers. They frequently report to be unable to play games, take part in school excursions, or social events because of being too slow (see letter of support, Section 7.3).

All HCPs who completed the related question in the dedicated online survey to gain feedback on ActiMyo® from the HCP perspective (N = 38; see Section 7.1), as well as most of solicited experts (see letters of support), acknowledged the value of measuring the maximal ambulation speed to assess the change (decline or improvement) in patients ability to walk (Figure 2). It has been extensively reported and demonstrated that the progressive loss of the ability to walk fast is predictive of loss of ambulation, one of the major milestones in DMD.[3] Most of the HCPs surveyed reported that a wearable device like ActiMyo® is useful to detect changes in walking ability. In addition, some clinician experts highlighted the level of precision of the SV95C, as measured with ActiMyo®, in their letters of support by mentioning publications and its previous qualification by the EMA.

Figure 2: Healthcare Professionals' Feedback – Online Survey Answer to Question # 15



Lastly, the impact of the qualification of SV95C in DMD for patients and for drug development in NMDs from different stakeholder perspectives has been summarized in the article Servais et al. 2021.[39]

3.2.1.2. Patient's Perspective

Patient and Family Feedback from the 2018 PPMD Annual Conference Report

In the 2018 PPMD Annual Conference report, out of 156 family members who responded, 11% identified the ability to participate in clinical trials without travelling large distances as one of the 2 greatest needs in the current clinical trial landscape. This suggests that the benefits of the SV95C as a home-based assessment rather than reliance on a test that requires repeated in-clinic visits would be

appreciated by patients and their families.

Patient and Caregiver Online Survey

A total of 549 patients with NMD (or their caregivers) answered the online survey (see Section 3.1.1) worldwide. Of these, 92 responses were from patients living with DMD and their families (mainly from the US; 14 patients and 78 caregivers). The report of the full data relating to ambulation from this survey can be found in Section 7.2. However, key findings relating to data from those with DMD are presented below. Data from those with other NMDs are presented separately in Section 4.2.1.

The survey data from those living with, or caring for someone with, DMD (N=92) confirms that ambulation is a key element of DMD from the patient/caregiver perspective. It is one of the first symptoms noticed and ambulation is associated with independence or freedom and the ability to get around. A lack of ambulation leads to a greater dependency from technology and others; this is also reported in ambulant patients. Additionally, ambulant patients reported other consequences of the disease, such as fatigue whilst moving around, fear of falling, and a limitation to activities (both for the individual with DMD and their family). Walking is an important aspect of ambulation for both ambulant and non-ambulant DMD patients, and it is the function that both groups would most like to see restored in a clinical trial. Most patients also indicated that a change in top speed of walking would represent an improvement in ambulation. The majority also indicated a preference for a wearable device to capture mobility in a clinical trial, reporting that a device such as ActiMyo® would make participating in a clinical trial more attractive, and that they would be willing to use it for as long as the trial lasts. This reveals that, for patients, the burden of wearing the device is less than the value of measuring ambulation precisely in a real-life setting.

The characteristics of those survey respondents either living with, or caring for someone with, DMD (the "DMD population") are presented in Table 11. This shows that a wide range of current age and age at which symptoms first appeared is represented, and there is an almost equal split between those who are currently ambulant (able to walk 10m (25ft) without help, based on survey response) and not. Table 12 summarizes the ages for the DMD patients represented in the survey, broken down by patient/caregiver and ambulatory status. As to be expected, ambulant patients were younger than non-ambulant, and most of them did not complete the survey themselves.

Table 11: Characteristics of Survey Respondents - DMD (N = 92)

		DMD (N = 92)
Age of patient (in years)	Mean (SD)	15.5 (9.47)
	Median	13.0
	Min – Max	1 – 57
	Q1, Q3	9.0, 21.0
Age symptoms first appeared (in years)	Mean (SD)	3.4 (2.48)
	Median	3.0
	Min – Max	0 – 12
	Q1, Q3	2.0, 4.0
	Missing	8
Ambulant[1]	Yes	49 (53.2%)
	No	43 (46.7%)
Relationship to patient	Caregiver	2 (2.6%)
	Father	14 (18.2%)
	Mother	56 (72.7%)
	Grandparents	4 (5.2%)
	Legal guardian	1 (1.3%)
	Missing[2]	15
Country	Belgium	1
	Germany	1
	United States of America (USA)	90

Source: Table adapted from Table 1 Survey Analysis Report

DMD = Duchenne's Muscular Dystrophy; Max = maximum; Min = minimum; N = number of subjects in the population; Q1 = first quartile (25th percentile); Q3 = third quartile (75th percentile); SD = standard deviation

[1]Ambulant defined as ability to walk 10m (25ft) without help, based on survey response.

[2]Question not answered by patients as respondents, only by caregivers.

Table 12: Patient Age by Patient/Caregiver Report and by Walking Ability - DMD

Age	Ambulant	Non-Ambulant
Patients	3 – 11.3 / 11.0 (0.58) [11 – 12]	11 – 31.4 / 28.0 (11.14) [18 – 57]
Caregivers	46 – 9.7 / 9.0 (4.20) [3 – 23]	32 – 18.6 / 17.0 (6.86) [0.7 – 36]
All	49 – 9.8 / 9.0 (4.1) [3 – 23]	43 – 21.9 / 21.0 (9.8) [0.7 – 57]

DMD = Duchenne muscular dystrophy; SD = standard deviation

Data are expressed as follows: N – Mean / Median (SD) [Min-Max]

The survey results are presented for the total DMD population and stratified by patients' ambulatory status as perspectives and experiences may be different for those patients who are still ambulant compared to those patients who have lost ambulation. Due to the few answers direct from the patient, a sub stratification of results by respondent is not presented. Therefore, the survey results are mostly reflecting caregiver opinions and perspectives on the patient experience, rather than those of patients directly.

The survey results confirm that loss of ambulation is an important milestone in DMD. Ambulation difficulties were the most commonly reported first symptom experienced in DMD (by 65.9%) and this included difficulties with walking, running or climbing stairs (see Table 13). Developmental delays were also amongst the first symptoms most commonly reported in DMD (35.2%).

Table 13: First Symptoms Experienced in DMD by Walking Ability

Symptom[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)
Missing	0	1	1
Ambulation difficulties	32 (65.3%)	28 (66.7%)	60 (65.9%)
Walking difficulties	*19 (38.8%)*	*21 (50.0%)*	*40 (44.0%)*
Stair difficulties	*11 (22.4%)*	*8 (19.0%)*	*19 (20.9%)*
Running difficulties	*9 (18.4%)*	*8 (19.0%)*	*17 (18.7%)*
Developmental delays	18 (36.7%)	14 (33.3%)	32 (35.2%)
Falling or clumsiness	14 (28.6%)	11 (26.2%)	25 (27.5%)
Getting up difficulties	6 (12.2%)	15 (35.7%)	21 (23.1%)
Large calves	8 (16.3%)	12 (28.6%)	20 (22.0%)
Muscle strength	9 (18.4%)	5 (11.9%)	14 (15.4%)
Fatigue	8 (16.3%)	1 (2.4%)	9 (9.9%)
CPK or liver enzymes elevated	3 (6.1%)	3 (7.1%)	6 (6.6%)
Gower's symptoms	2 (4.1%)	4 (9.5%)	6 (6.6%)

Symptom[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)
Mobility limited	2 (4.1%)	3 (7.1%)	5 (5.5%)
Speech difficulties	3 (6.1%)	2 (4.8%)	5 (5.5%)
Pain	3 (6.1%)	1 (2.4%)	4 (4.4%)
Distal impacts noticed (e.g., ability to ride a bike)	1 (2.0%)	3 (7.1%)	4 (4.4%)

CPK = creatine phosphokinase; DMD = Duchenne muscular dystrophy

Results are expressed in Number(Percentage excluding missing answers)

Survey participants were asked to write in an open text box what mobility meant to them. Results from thematically analyzing these responses for patients with DMD (Table 14) show that, overall, mobility often meant an ability to move about (31.8%), having independence or freedom (29.4%), ambulation (28.2%) or getting around from place to place (21.2%). Walking was the activity most often referred to under ambulation; for non-ambulant patients this was the only activity referred to, however for ambulant patients running and climbing stairs were also noted.

Table 14: Meaning of Mobility in DMD by Walking Ability

Meaning of Mobility[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)
Missing	4	3	7
Ability to move	12 (26.7%)	15 (37.5%)	27 (31.8%)
Independence or freedom	13 (28.9%)	12 (30.0%)	25 (29.4%)
Ambulation	16 (35.6%)	8 (20.0%)	24 (28.2%)
Walking	*15 (33.3%)*	*8 (20.0%)*	*23 (27.1%)*
Running	*2 (4.4%)*	*0*	*2 (2.4%)*
Stairs	*1 (2.2%)*	*0*	*1 (1.2%)*
Getting around	7 (15.6%)	11 (27.5%)	18 (21.2%)
Ability to do daily functions	4 (8.9%)	1 (2.5%)	5 (5.9%)
Wheelchair use	1 (2.2%)	3 (7.5%)	4 (4.7%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple meanings. Meanings are not mutually exclusive, so column percentages may total more than 100%.

Percentages reported are calculated based on the number of non-missing participants in the column.

When asked how DMD impacted their mobility, walking was most mentioned (by 29.4% of the DMD population as a whole), by both ambulant and non-ambulant DMD patients (Table 15). A review of the survey responses indicated that walking in DMD was impacted in a number of ways: ability (no longer being able to walk), speed (having to walk slower), distance (not being able to walk as far), and time

(not being able to walk for as long at time). Both ambulant and non-ambulant patients with DMD also noted how DMD led to greater reliance on technology such as a wheelchair or walker/Zimmer frame or greater reliance on other people, although both of these were more commonly reported for non-ambulant patients with DMD (45.0% versus 6.7% and 20.0% versus 6.7%, respectively).

Table 15: Impact on Mobility in DMD by Walking Ability

Impact on Mobility[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DM (n = 92)
Missing	4	3	7
Walking impacted	11 (24.4%)	14 (35.0%)	25 (29.4%)
Reliance on technology	3 (6.7%)	18 (45.0%)	21 (24.7%)
Reliance on others	3 (6.7%)	8 (20.0%)	11 (12.9%)
Weakness	6 (13.3%)	5 (12.5%)	11 (12.9%)
Upper body mobility or strength	0	10 (25.0%)	10 (11.8%)
Social impacts	7 (15.6%)	1 (2.5%)	8 (9.4%)
Household duties or everyday tasks	4 (8.9%)	3 (7.5%)	7 (8.2%)
Limits physical activities	4 (8.9%)	1 (2.5%)	5 (5.9%)
No Impact	4 (8.9%)	1 (2.5%)	5 (5.9%)
Falling or fear of falling	4 (8.9%)	0	4 (4.7%)
Decline in mobility	3 (6.7%)	1 (2.5%)	4 (4.7%)
Fatigue	4 (8.9%)	0	4 (4.7%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple impacts. Impacts are not mutually exclusive, so column percentages may total more than 100%.

Percentages reported are calculated based on the number of non-missing participants in the column.

The survey results also demonstrated that DMD has a substantial impact upon family activities and that ambulation is a key part of this (Table 16). When asked about how DMD impacted upon family activities, needing to plan ahead and activities being limited or altered were the 2 most commonly reported impacts overall (35.3% and 30.8%, respectively). These were the most common for both ambulant and non-ambulant patients with DMD, although ambulant patients were more likely to report activities being limited or altered than non-ambulant. Walking, hiking, running, or similar outdoor activities were the activities most often noted as being limited or altered for ambulant patients with DMD (26.7%), whereas non-ambulant patients with DMD more often reported having limited options for family activities (15.0%). Non-ambulant patients with DMD were also more likely to report having their travel impacted (10.0%) and doing fewer activities outside the home (12.5%) than ambulant patients with DMD (4.4% and 2.2%, respectively).

Table 16: Impact on Family Activities in DMD by Walking Ability

Impact on Family Activities[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)
Missing	4	3	7
Activities limited or altered	20 (44.4%)	10 (25.0%)	30 (35.3%)
Walking, hiking, running, other outdoor activities	*12 (26.7%)*	*2 (5.0%)*	*14 (16.5%)*
Limited options	*3 (6.7%)*	*6 (15.0%)*	*9 (9.9%)*
Planning ahead needed	15 (33.3%)	13 (32.5%)	28 (30.8%)
No Impact	6 (13.3%)	2 (5.0%)	8 (8.8%)
Travel impacted	2 (4.4%)	4 (10.0%)	6 (6.6%)
Less activity outside home	1 (2.2%)	5 (12.5%)	6 (6.6%)

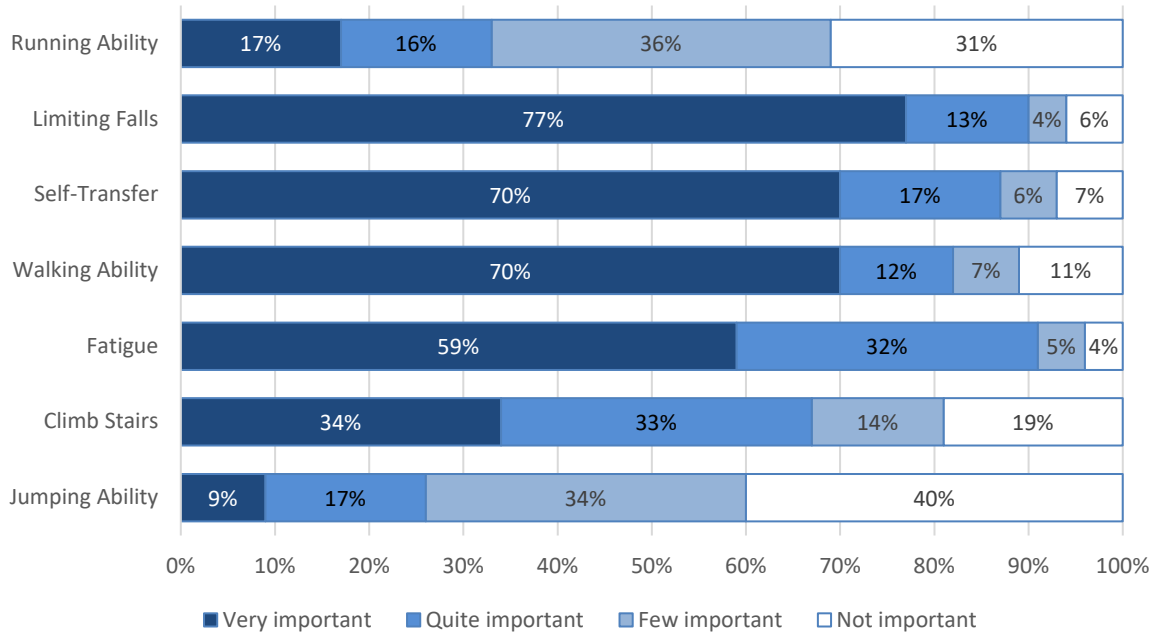
DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple impacts. Impacts are not mutually exclusive, so column percentages may total more than 100%.

Percentages reported are calculated based on the number of non-missing participants in the column.

When looking at which aspects of ambulation are most important to patients and caregivers in DMD, the results clearly show that the aspects most reported to be "very important" were limiting falls (77%), the ability to self-transfer (70%) and walking (70%). When the results were broken down by ambulatory status, walking ability was the most important aspect by far, being rated as "very important" by 86% ambulant patients, "quite important" by a further 12%, and the remaining 2% rated it as "few important". Thus, 100% of the ambulant DMD population considered walking to be important at some level. Although not as commonly rated as being "very" important, walking ability was still an important aspect of ambulation for the majority of non-ambulant patients, with 77% non-ambulant patients rating this as either few/quite/very important. These findings are illustrated in Figure 3 and Figure 4.

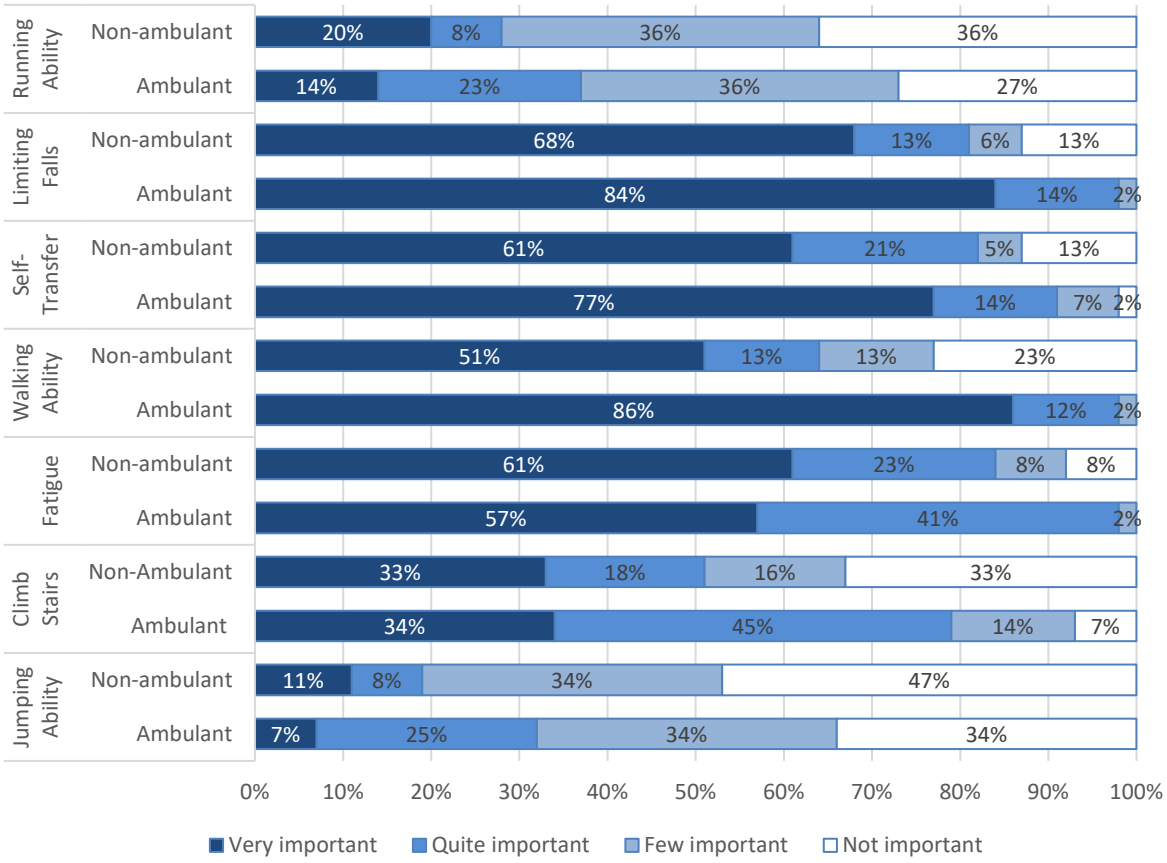
Figure 3: Aspects of Ambulation - Importance Ratings of DMD Population



DMD = Duchenne muscular dystrophy

Percentages based off n = 82 DMD participants who answered these questions.

Figure 4: Aspects of Ambulation - Importance Ratings of DMD Population by Walking Ability



DMD = Duchenne muscular dystrophy

Percentages based off n = 44 ambulant and n=38 non-ambulant DMD participants who answered these questions.

When considering a treatment for DMD, patients and caregivers most commonly reported that they expect a treatment to at least slow down or prevent the progression of the disease (Table 17). This was observed for both ambulant and non-ambulant patients. Improvements in muscle strength, general condition and mobility were also expectations reported in both ambulant and non-ambulant DMD populations.

Table 17: Expectations for Clinical Trial in DMD by Walking Ability

Expectation[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	DMD (n = 92)
Missing	6	5	11
Slow or stop disease progression	21 (48.8%)	16 (42.1%)	37 (45.7%)
Muscle strength improved	7 (16.3%)	6 (15.8%)	13 (16.0%)
General improvement	5 (11.6%)	7 (18.4%)	12 (14.8%)
Mobility improved	5 (11.6%)	1 (2.6%)	6 (7.4%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple expectations. Expectations are not mutually exclusive, so column percentages may total more than 100%.

Percentages reported are calculated based on the number of non-missing participants in the column.

Ambulation, particularly walking, is the key function that most ambulant DMD patients would like to see maintained in a clinical trial. Not surprisingly, as those who are non-ambulant have already lost this ability, these respondents focused more upon cardiovascular or pulmonary function, upper body function, muscle strength, and hand function (Table 18).

Table 18: Function to Maintain in DMD by Walking Ability

Function to Maintain[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)
Missing	7	11	18
Ambulation	23 (54.8%)	1 (3.1%)	24 (32.4%)
Walking	*19 (45.2%)*	*1 (3.1%)*	*20 (27.0%)*
Stairs	*1 (2.4%)*	*0*	*1 (1.4%)*
Cardiovascular or pulmonary	8 (19.0%)	11 (34.4%)	19 (25.7%)
Muscle strength	3 (7.1%)	5 (15.6%)	8 (10.8%)
Upper body function	1 (2.4%)	6 (18.8%)	7 (9.5%)
Hand function	0	5 (15.6%)	5 (6.8%)
Mobility	3 (7.1%)	1 (3.1%)	4 (5.4%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple functions. Functions are not mutually exclusive, so column percentages may total more than 100%.

Percentages reported are calculated based on the number of non-missing participants in the column.

In terms of functions that respondents would most want to see improved in a clinical trial, ambulant

DMD patients again focused mostly upon ambulation, referring to walking in addition to climbing stairs and running. Non-ambulant patients again focused upon muscle strength and cardiovascular or pulmonary function (Table 19).

Table 19: Function to be Improved in DMD by Walking Ability

Function to be Improved[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)[2]
Missing	9	8	17
Muscle strength	10 (25.0%)	12 (34.3%)	22 (29.3%)
Ambulation	12 (30.0%)	1 (2.9%)	13 (17.3%)
Walking	*4 (10.0%)*	*1 (2.9%)*	*5 (6.7%)*
Stairs	*5 (12.5%)*	*0*	*5 (6.7%)*
Running	3 (7.5%)	0	3 (4.0%)
Cardiovascular or pulmonary	4 (10.0%)	7 (20.0%)	11 (14.7%)
Fatigue or energy levels	5 (12.5%)	2 (5.7%)	7 (9.3%)
Mobility	2 (5.0%)	2 (5.7%)	4 (5.3%)
Upper limb function	0	4 (11.4%)	4 (5.3%)
Daily functions	2 (5.0%)	2 (5.7%)	4 (5.3%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple functions. Functions are not mutually exclusive, so column percentages may total more than 100%.

[2]Percentages reported are calculated based on the number of non-missing participants in the column.

However, in both the ambulant and non-ambulant DMD populations, ambulation was a function they most wanted to see restored. For those who are ambulant, the focus was upon walking, climbing stairs and running, and for those who are non-ambulant, the focus was solely on walking (Table 20). Outcomes related to the distance walked (either before stopping or per day; n = 36 in total out of 62 DMD respondents answering this question) were considered to best represent an improvement in ambulation, along with the experience of fatigue during ambulation (n = 31). (See Table 21).

Interestingly, ability to walk fast appeared not representing such an improvement in ambulation for the overall DMD population, but all 3 ambulant patients with DMD who answered the survey reported that ability to walk fast represented an improvement in ambulation. When considering all ambulant NMDs patients who answered the survey, including DMD, ability to walk fast appeared to be the second item that represented the best an ambulation improvement (42% in patients, vs 8% in caregivers). In contrary, the number of falls per day appeared to be the third best representative of an improvement for caregivers (49% in caregivers vs 8% in patients), suggesting that caregivers were probably more concern about the risk of falls that might result in dramatic consequences. (See results presented at the World Muscle Society Congress 2021, Poster EP322, Section 7.2)

Table 20: Function to be Restored in DMD by Walking Ability

Function to Restore[1]	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total DMD (n = 92)[2]
Missing	11	9	20
Ambulation	10 (26.3%)	11 (32.4%)	21 (29.2%)
Walking	*2 (5.3%)*	*10 (29.4%)*	*12 (16.7%)*
Stairs	*4 (10.5%)*	*0*	*4 (5.6%)*
Running	*3 (7.9%)*	*0*	*3 (4.2%)*
Muscle strength	8 (21.1%)	8 (23.5%)	16 (22.2%)
Fatigue or energy levels	9 (23.7%)	0	9 (12.5%)
Quality of life	3 (7.9%)	1 (2.9%)	4 (5.6%)

DMD = Duchenne muscular dystrophy

[1]Participants' responses could include multiple functions. Functions are not mutually exclusive, so column percentages may total more than 100%.

[2]Percentages reported are calculated based on the number of non-missing participants in the column.

Table 21. Aspects that Best Represent Improvement in Ambulation - DMD by Walking Ability

Best represents an ambulation improvement*	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total (n = 92)
Not concerned with ambulation outcomes	13	17	30
Fatigue during ambulation	18 (50.0%)	13 (50.0%)	31 (50.0%)
Distance walked before stopping	16 (44.4%)	9 (34.6%)	25 (40.3%)
Number of falls per day	14 (38.9%)	10 (38.5%)	24 (38.7%)
Ability to climb stairs	13 (36.1%)	2 (7.7%)	15 (24.2%)
Ease in climbing stairs (both feet on each step or one foot per step)	11 (30.6%)	1 (3.8%)	12 (19.4%)
Distance walked per day	6 (16.7%)	5 (19.2%)	11 (17.7%)
Time measured to climb stairs	4 (11.1%)	0	4 (6.5%)
Ability to walk fast	2 (5.6%)	1 (3.8%°	3 (4.8%)
Other[1]	0	3 (11.5%)	3 (4.8%)
Prefer not to respond	0	2 (7.7%)	2 (3.2%)

DMD = Duchenne muscular dystrophy; NMD = neuromuscular disease

*Due to the ability for participants to check all that apply, percentages are not calculated in SAS output; [1] 'Ability to walk in general', 'Ability to walk, sit up from reclined unassisted, groom and dress

independently', and 'Being able to stand up'

When specifically asked whether measuring a change in the top speed while walking is representative of an ambulation improvement[g], overall, 57.78% of those with DMD who responded agreed, and this agreement was found for both ambulant and non-ambulant DMD patients. Considering only answers from ambulant patients with NMDs, including DMD (N = 119), 84% of patients agreed that measuring a change in the top speed while walking was representative of an ambulation improvement (see Table 22 and Section 7.2, poster WMS2021). Estimates given on the distance that could currently be walked in 6-minutes showed that there was a range of ambulatory function represented within the survey population. When asked what they would consider to be an improvement in ambulation, almost half of the respondents reported that an improvement in 5 to 10 meters walking distance over 6 minutes represented an improvement, and 70% rated 20 to 40 meters as an improvement (see Table 22).

Table 22: Walking Speed as Improvement in the DMD Population by Walking Ability

Best Represents an Ambulation Improvement*		Ambulant (n = 49)	Non-Ambulant (n = 43)	Total (n = 92)
Change in top speed as ambulation improvement	Missing	21	26	47
	Yes	15 (53.57%)	11 (64.71%)	26 (57.78%)
	No	12 (42.86%)	5 (29.41%)	17 (37.78%)
	Prefer not to respond	1 (3.57%)	1 (5.88%)	2 (4.44%)
Distance walked in 6 minutes	n missing	20	25	45
	less than 150 meters // 500 feet	2 (6.90%)	0	2 (4.26%)
	150 to 300 meters // 500 to 1000 feet	6 (20.69%)	0	6 (12.77%)
	300 to 450 meters // 1000 to 1500 feet	8 (27.59%)	0	8 (17.02%)
	450 to 600 meters // 1500 to 2000 feet	2 (6.90%)	0	2 (4.26%)
	I don't know	11 (37.93%)	3 (16.67%)	14 (29.79%)
	I prefer not to respond	0	4 (22.22%)	4 (8.51%)
	It is too difficult for him/her to walk during 6 minutes	0	9 (50.00%)	9 (19.15%)
	It is too difficult for me to walk during 6 minutes	0	2 (11.11%)	2 (4.26%)
	n missing	23	26	49

[g] "Do you think that measuring a change in the top speed while walking is representative of an ambulation improvement?"

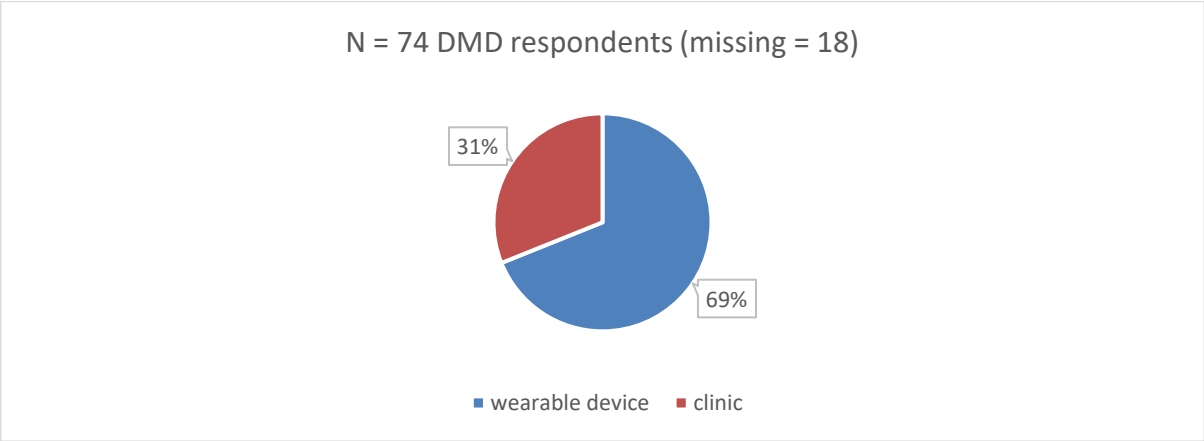
Best Represents an Ambulation Improvement*		Ambulant (n = 49)	Non-Ambulant (n = 43)	Total (n = 92)
5 to 10 meters // 15 to 30 feet considered as improvement?	Improvement	2 (7.69%)	8 (47.06%)	10 (23.26%)
	Acceptable improvement	9 (34.62%)	1 (5.88%)	10 (23.26%)
	Unacceptable improvement	11 (42.31%)	0	11 (25.58%)
	Not applicable	4 (15.38%)	8 (47.06%)	12 (27.91%)
20 to 40 meters // 60 to 120 feet considered as improvement?	n missing	24	28	52
	Improvement	10 (40.00%)	4 (26.67%)	14 (35.00%)
	Acceptable improvement	12 (48.00%)	2 (13.33%)	14 (35.00%)
	Unacceptable improvement	1 (4.00%)	0	1 (2.50%)
	Not applicable	2 (8.00%)	9 (60.00%)	11 (27.50%)
50 to 100 meters // 150 to 300 feet considered as improvement?	n missing	26	28	54
	Improvement	20 (86.96%)	4 (26.67%)	24 (63.16%)
	Acceptable improvement	2 (8.70%)	2 (13.33%)	4 (10.53%)
	Unacceptable improvement	0	0	0
	Not applicable	1 (4.35%)	9 (60.00%)	10 (26.32%)

DMD = Duchenne muscular dystrophy

Results are expressed in Number(Percentage excluding missing answers)

When asked about their preferences for how to have mobility assessed during a clinical trial, the majority of the DMD population, both ambulant and non-ambulant, indicated that they would prefer to use a wearable device in a real world setting rather than a regular assessment by a physiotherapist or clinician in a clinic-based setting (Figure 5). Among those within the DMD population who indicated they would prefer a device and provided an explanation (n = 7), participants generally noted that they felt a device would provide more accurate data (n = 4). Among those who indicated they would prefer a physiotherapist or physician to make regular assessments and provided an explanation (n = 7), participants typically noted they either did not like the device size (n = 3) or would prefer a combination of both a device and physician (n = 2). The survey participants had been shown a picture of the ActiMyo® device and only 1 DMD participant had actually used it previously (Table 23).

Figure 5: Illustration of Preference for Method of Assessing Mobility in a Clinical Trial - DMD



DMD = Duchenne muscular dystrophy

Reflecting the overall preference for a wearable device, over 70% (n = 55) of those DMD participants who responded to the survey questions around clinical trials indicated that such a device as ActiMyo® would make participating in a clinical trial more attractive and over 75% reported they would be willing to use ActiMyo®. Finally, 44.6% of DMD participants indicated they would be willing to wear ActiMyo® continuously for as long as the trial lasted. However, several also noted that they did not know how long they would wear it. It is also important to note that many participants (n ≥ 14) did not answer these questions about ActiMyo® (Table 23).

Table 23: Feedback on ActiMyo® Device in DMD by Walking Ability

Question	Response	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total (n = 92)
Prior use of ActiMyo®	Missing	7	7	14
	No	41 (97.62%)	36 (100.0%)	77 (98.7%)
	Yes	1 (2.38%)	0	1 (1.9%)
Would device such as ActiMyo® make participating in clinical trials more attractive	n missing	7	7	14
	No	14 (33.33%)	7 (19.44%)	21 (26.9%)
	Yes	28 (66.67%)	27 (75.00%)	55 (70.5%)
	I prefer not to respond	0	2 (5.56%)	2 (2.6%)
Willing to use ActiMyo®	n missing	7	7	14
	No	0	2 (5.56%)	2 (2.6%)
	Yes	35 (83.33%)	26 (72.22%)	61 (78.2%)
	I don't know	7 (16.67%)	7 (19.44%)	14 (18.0%)
How long willing to wear ActiMyo®	n missing	7	11	18
	As long as the trial lasts	13 (30.95%)	20 (62.50%)	33 (44.6%)
	2 weeks	5 (11.90%)	1 (3.13%)	6 (8.1%)

Question	Response	Ambulant (n = 49)	Non-Ambulant (n = 43)	Total (n = 92)
	1 month	3 (7.14%)	2 (6.25%)	5 (6.8%)
	6 months	5 (11.90%)	0	5 (6.8%)
	1 year or more	2 (4.76%)	1 (3.13%)	3 (4.1%)
	I don't know	14 (33.33%)	8 (25.00%)	22 (29.7%)
Most important limitation to wearing ActiMyo®	n missing	7	7	14
	Tolerability / Discomfort of wearing the device	22 (52.38%)	18 (50.00%)	40 (51.3%)
	Size *and* weight of the device	12 (28.57%)	5 (13.89%)	17 (21.8%)
	The appearance of the device	3 (7.14%)	1 (2.78%)	4 (5.1%)
	The device is not waterproof	1 (2.38%)	3 (8.33%)	4 (5.1%)
	Duration of having to wear the device	2 (4.76%)	2 (5.56%)	4 (5.1%)
	Size *or* weight of the device	0	3 (8.33%)	3 (3.9%)
	Looking different because you are wearing the device	1 (2.38%)	1 (2.78%)	2 (2.6%)
	No limitation	1 (2.38%)	3 (8.33%)	4 (5.1%)

DMD = Duchenne muscular dystrophy

Results are expressed in number (percentage excluding missing answers)

3.2.2. Quantitative Evidence

3.2.2.1. Population

The number of patients and control subjects for each study is provided in Table 1. The population characteristics, ActiMyo® configuration, and recording periods used in each clinical study are listed in Table 4. The median age in years of patients and control subjects was 8.0 and 9.3, respectively, ranging from 5 to 14 years, and 6 to 14.2 years. The mean and median age, and the age range for each study is provided in Table 24.

Table 24: Age Characteristics in Global, for Each Study, and Used to Study the Natural Course of the Disease (NHS) and the Response to Corticosteroids (TTT)

Age (Years)	N	Mean	Median	SD	Min	Max	P-value*
DMD	125	8.1	8.0	1.94	5.0	14.0	< 0.001
CTRL	66	9.6	9.3	2.16	6.0	14.2	
DMD in NHS	107	8.254	8.000	1.9314	5.0	14.0	0.011
DMD starting TTT	18	6.991	6.457	1.6638	5.0	10.0	
DMD in CT-A	34	8.7	8.2	2.13	6.0	13.7	0.005
DMD in CT-B	51	8.4	8.0	1.53	6.0	11.0	
DMD in CT-C	7	7.7	8.0	1.98	5.0	10.0	
DMD In clinic	7	7.0	6.5	1.48	5.1	9.7	
DMD in NHS-A	2	5.5	5.5	0.71	5.0	6.0	
DMD in NHS-B	13	7.2	6.0	2.54	5.0	14.0	
DMD in NHS-C	11	7.1	6.8	1.57	5.2	10.0	
CTRL in NHS-A	62	9.6	9.3	2.13	6.0	14.2	-
CTRL in NHS-B	4	9.3	8.5	2.87	7.0	13.0	

CTRL = control population; DMD = Duchenne muscular dystrophy; NHS = Natural history study; SD = standard deviation; TTT = treatment

* Independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-Wallis Test (when more than 2 samples)

3.2.2.2. Test-retest Reliability

Based on measures performed 1 month apart in 2 successive recording periods for 52 DMD patients, the ICC coefficients were high (0.970) and plots homogenously distributed on the Bland and Altman graph, indicating excellent reliability between the 2 measures whatever the SV95C value (Table 25 and Figure 6).

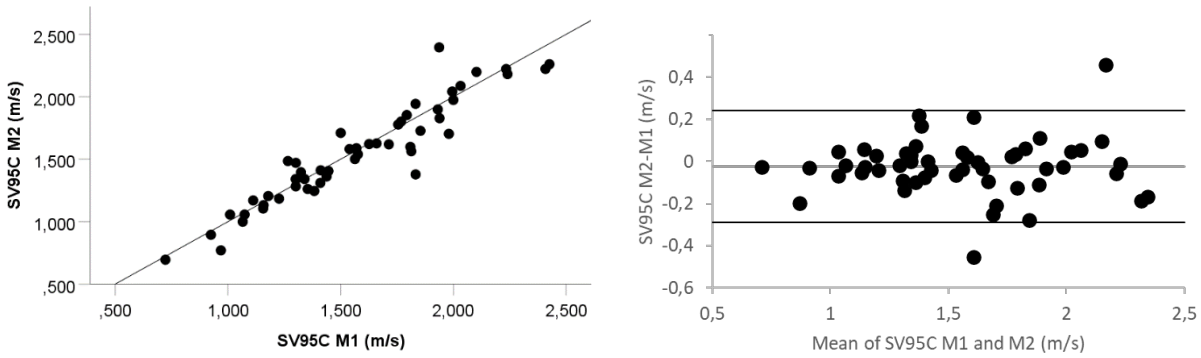
Table 25: Test-Retest Reliability of the SV95C - Intra-class Correlation Coefficient

	N	ICC	95% CI
DMD	52	0.970	[0.947 – 0.983]

CI = confidence interval; DMD = Duchenne muscular dystrophy; ICC = intra-class correlation coefficient; SV95C = 95th centile of the stride velocity

Figure 6: Comparison of SV95C Measured 1 Month Apart in 2 Successive Recording Periods

A **B**



A. SV95C measured in month 1 vs month 2. B. Bland and Altman representation, grey line represents the mean of the difference between SV95C measured at month 2 and month 1 (mean SV95C difference = -0.024m/s), dark lines represent the mean difference between the measurements ± 1.96 SD (with SD = 0.136m/s).

3.2.2.3. Construct Validity

3.2.2.3.1. Known-groups Validity

To confirm the known-groups validity of the SV95C, the DMD population was compared with an age-matched healthy control population at Baseline. Overall, the results verified that SV95C was able to discriminate patients with DMD from the healthy control subjects, with lower median SV95C scores reported for patients in the DMD population (1.563 m/s) compared with the healthy control population (2.713 m/s; P-value < 0.001; Table 26). As expected, no statistical significance (P-values > 0.05) was observed between DMD patients who participated in Studies CT-A, CT-B, CT-C, NHS-A, NHS-B, NHS-C, and in clinic DMD patients or between the DMD population used to study the natural course of the disease versus DMD populations starting corticosteroids. Based on the low sample size of healthy control subjects in Study NHS-B, a comparison between the healthy control subjects in the Studies NHS-A and NHS-B was not performed (Table 26).

Table 26: Comparison of SV95C Scores Between the DMD and Healthy Control Populations, Different Studies, and the DMD NHS Versus TTT Populations at Baseline

SV95C (m/s)	N	Mean	Median	SD	Min	Max	P-value*
DMD	**125**	**1.571**	**1.563**	**0.3818**	**0.700**	**2.500**	**< 0.001**
CTRL	**66**	**2.621**	**2.713**	**0.4578**	**1.500**	**3.600**	
DMD_NHS	107	1.588	1.580	0.3825	0.7	2.5	0.226
DMD_TTT	18	1.474	1.484	0.3733	0.9	2.3	
CT-A-DMD	34	1.620	1.573	0.3537	1.100	2.400	0.828
CT-B-DMD	51	1.599	1.600	0.3785	0.800	2.500	
CT-C-DMD	7	1.505	1.489	0.4984	0.9	2.3	
In clinic-DMD	7	1.419	1.480	0.3048	1.1	1.8	
NHS-A-DMD	2	1.682	1.682	0.3368	1.4	1.9	
NHS-B-DMD	13	1.480	1.411	0.4124	0.9	2.4	
NHS-C-DMD	11	1.519	1.539	0.4588	0.7	2.0	
NHS-A-CTRL	62	2.673	2.726	0.4034	1.6	3.6	-
NHS-B-CTRL	4	1.819	1.574	0.5647	1.5	2.7	

CTRL = control; DMD = Duchenne muscular dystrophy; SD = standard deviation; SV95C = 95th centile of the stride velocity; TTT = treatment

*Independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-Wallis Test (when more than 2 samples).

Similar results were observed for the existing COA where the median 6MWD score was significantly lower in the DMD population compared with the healthy control population (389.0m versus 605.0m, respectively; P-value < 0.001) and higher (requiring more time to climb 4 stairs) for the 4SC (3.40 seconds versus 1.27 seconds; P-value < 0.001). Of note, for the 4SC, 3 healthy control subjects were compared against the DMD population (n = 109). A larger sample of healthy control subjects would be needed to confirm these results. As expected, no statistical significance (P-values > 0.05) was observed between DMD patients in the different studies (CT and NHS studies) or between the DMD NHS versus TTT populations. A comparison between the healthy control subjects in Studies NHS-A and NHS B was also not performed due to low sample size (Table 27 and Table 28).

Table 27: Comparison of 6MWD Between the DMD and Healthy Control Populations, Different Studies, and the DMD NHS Versus TTT Populations at Baseline

6MWD (m)	N	Mean	Median	SD	Min	Max	P-value*
DMD	**109**	**389.4**	**389.0**	**75.6**	**25.0**	**512.0**	**< 0.001**
CTRL	**63**	**606.7**	**605.0**	**63.5**	**464.0**	**761.0**	
CT-A-DMD	34	414.5	426.3	56.2	290.0	512.0	0.134

6MWD (m)	N	Mean	Median	SD	Min	Max	P-value*
CT-B-DMD	50	384.7	386.0	82.3	25.0	510.3	
CT-C-DMD	-	-	-	-	-	-	
In clinic-DMD	-	-	-	-	-	-	
NHS-A-DMD	2	354.5	354.5	85.6	294.0	415.0	
NHS-B-DMD	12	363.6	355.0	52.6	290.0	475.0	
NHS-C-DMD	11	367.6	395.5	102.8	151.0	492.5	
NHS-A-CTRL	62	606.9	605.5	64.0	464.0	761.0	-
NHS-B-CTRL	1	-	-	-	-	-	
DMD_NHS	105	391.0	392.0	76.1	25.0	512.0	0.114
DMD_TTT	4	347.3	340.0	51.6	294.0	415.0	

6MWD = 6-minute walking distance; CTRL = control; DMD = Duchenne muscular dystrophy; NHS = natural history study; SD = standard deviation; TTT = Treatment

*Independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-Wallis Test (when more than 2 samples)

Table 28: Comparison of 4SC Between the DMD and Healthy Control Populations, Different Studies, and the DMD NHS Versus TTT Populations at Baseline

4SC (s)	N	Mean	Median	SD	Min	Max	P-value*
DMD	**109**	**3.86**	**3.40**	**1.63**	**1.29**	**8.70**	**< 0.001**
CTRL	**3**	**1.32**	**1.27**	**0.17**	**1.19**	**1.51**	
CT-A-DMD	34	3.63	3.40	1.50	1.70	8.70	0.052
CT-B-DMD	51	3.90	3.40	1.56	1.62	8.00	
CT-C-DMD	-	-	-	-	-	-	
In clinic-DMD	-	-	-	-	-	-	
NHS-A-DMD	-	-	-	-	-	-	
NHS-B-DMD	13	4.90	4.93	2.06	1.29	8.57	
NHS-C-DMD	11	3.12	2.80	1.33	1.90	6.69	
NHS-A-CTRL	-	-	-	-	-	-	-
NHS-B-CTRL	3	1.32	1.27	0.17	1.19	1.51	
DMD_NHS	107	3.83	3.40	1.64	1.29	8.70	0.136
DMD_TTT	2	5.05	5.05	0.28	4.85	5.25	

4SC = 4-stair climb test; CTRL = control; DMD = Duchenne muscular dystrophy; NHS = natural history study; SD = standard deviation; TTT = Treatment

*Independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-

Wallis Test (when more than 2 samples)

Very few CTRL performed the 4SC test. There will be more later with ActiLiège-Next study.

The NSAA was not performed by healthy subjects. As expected, no statistical significance (P > 0.05) was observed between DMD patients in the CT-A, CT-B, NHS-B, and NHS-C studies or between the DMD NHS versus TTT populations (Table 29).

Table 29: Comparison of NSAA Between the DMD and Healthy Control Populations, Different Studies, and the DMD NHS Versus TTT Populations at Baseline

NSAA (#)	N	Mean	Median	SD	Min	Max	P-value
DMD	109	22.8	23	6.28	2	33	-
CT-A-DMD	34	24.12	24.00	5.086	13	33	0.518
CT-B-DMD	51	22.43	22.00	6.792	2	33	
CT-C-DMD	-	-	-	-	-	-	
In clinic-DMD	-	-	-	-	-	-	
NHS-A-DMD	-	-	-	-	-	-	
NHS-B-DMD	13	23.69	23.00	5.202	18	33	
NHS-C-DMD	11	19.64	23.00	7.698	8	29	
DMD_NHS	107	22.87	23.00	6.325	2	33	0.530
DMD_TTT	2	20.50	20.50	3.536	18	23	

CT = Clinical trial; DMD = Duchenne muscular dystrophy; NHS = natural history study; NSAA = North Star Ambulatory Assessment; SD = standard deviation; TTT = Treatment

*Independent-Samples Mann-Whitney U Test (when 2 samples) or Independent-Samples Kruskal-Wallis Test (when more than 2 samples)

NSAA was not performed by healthy subjects. Ceiling effect (max. score = 34).

Furthermore, on stratifying by age group, comparisons between the DMD and healthy control groups in both the younger population (patients aged 5 to 7 years in the DMD group and 6 to 7 years in the control group), and the older population (patients aged 8 to 14 years in both the DMD and healthy control groups), SV95C and 6MWD were statistically lower in the DMD population compared with the healthy control population (P-values < 0.001; Table 30).

Table 30: Age Range Effect on SV95C, 6MWD, NSAA, 4SC (Comparison Between DMD and Control Groups)

Age Range	N	Mean	Median	SD	Min	Max	P-value*
Age (y)							
DMD [5 – 7]	57	6.4	6.6	0.8	5.0	7.9	0.015
CTRL [6 – 7]	17	7.0	7.0	0.65	6.0	8.0	
DMD [8 - 14]	68	9.5	9.0	1.47	8.0	14.0	0.001

CTRL [8 – 14]	49	10.5	10.2	1.7	8.0	14.2	
SV95C (m/s)							
DMD [5 – 7]	57	1.723	1.680	0.346	1.009	2.426	**< 0.001**
CTRL [6 – 7]	17	2.627	2.673	0.351	1.478	3.074	
DMD [8 - 14]	68	1.444	1.386	0.366	0.723	2.470	**< 0.001**
CTRL [8 – 14]	49	2.619	2.742	0.493	1.474	3.556	
6MWD (m)							
DMD [5 – 7]	47	401.1	407.0	60.3	247.0	507.0	**< 0.001**
CTRL [6 – 7]	16	549.5	564.5	45.9	464.0	602.0	
DMD [8 - 14]	62	380.5	373.5	84.9	25.0	512.0	**< 0.001**
CTRL [8 – 14]	47	626.2	625.0	56.7	489.0	761.0	

6MWD = 6-minute walking distance; CTRL = Control population; DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

* Independent-Samples Mann-Whitney U Test

When stratified by age group, a statistical difference was observed between the youngest (patients aged 5 to 7 years) and oldest DMD population (8 to 14 years) for both the SV95C and 4SC (P-values < 0.001 and 0.005, respectively; Table 31). This indicates that, as expected by the knowledge of the natural course of the disease, DMD patients in the older population had a lower stride velocity and took longer to climb 4 stairs compared with the younger population (median SV95C: 1.39 m/s versus 1.68, respectively; median 4SC: 3.75 seconds versus 3.06 seconds). No statistical significance was observed for the 6MWD and NSAA between age groups for the DMD population meaning that 6MWD and NSAA are less sensitive to characterize difference between younger and older patients. When comparing age groups for the control population (6 to 7 years of age versus 8 to 14 years of age), as expected, the only statistical significance was observed for the 6MWD (the distance walked as fast as possible in 6 minutes was higher in the older population [625.0 meters] versus the younger population [564.5 meters]; P-value < 0.001; Table 31).[40]

Overall, in children, growth and disease progression are confounding factors and SV95C appears more sensitive than 6MWD or NSAA to detect difference induced by the disease.

Table 31: Age Range Effect on SV95C, 6MWD, NSAA, 4SC (Comparison Within the DMD and Control Groups)

Age Range	N	Mean	Median	SD	Min	Max	P-value*
Age (y)							
DMD [5 – 7]	57	6.4	6.6	0.8	5.0	7.9	< 0.001
DMD [8 - 14]	68	9.5	9.0	1.5	8.0	14.0	
CTRL [6 – 7]	17	7.0	7.0	0.6	6.0	8.0	< 0.001
CTRL [8 – 14]	49	10.6	10.2	1.7	8.0	14.2	
SV95C (m/s)							
DMD [5 – 7]	57	1.723	1.680	0.3459	1.009	2.426	**< 0.001**
DMD [8 - 14]	68	1.444	1.386	0.3656	0.723	2.470	
CTRL [6 – 7]	17	2.627	2.673	0.3511	1.478	3.074	0.514
CTRL [8 – 14]	49	2.619	2.742	0.4926	1.474	3.556	
6MWD (m)							
DMD [5 – 7]	47	401.1	407.0	60.3	247.0	507.0	0.264
DMD [8 - 14]	62	380.5	373.5	84.9	25.0	512.0	
CTRL [6 – 7]	16	549.5	564.5	45.9	464.0	602.0	**< 0.001**
CTRL [8 – 14]	47	626.2	625.0	56.7	489.0	761.0	
NSAA (#)							
DMD [5 – 7]	47	23.96	23.00	4.800	10	33	0.107
DMD [8 - 14]	62	21.97	22.00	7.126	2	33	
4SC (s)							
DMD [5 – 7]	47	3.38	3.06	1.42	1.29	8.57	**0.005**
DMD [8 - 14]	62	4.21	3.75	1.70	1.70	8.70	

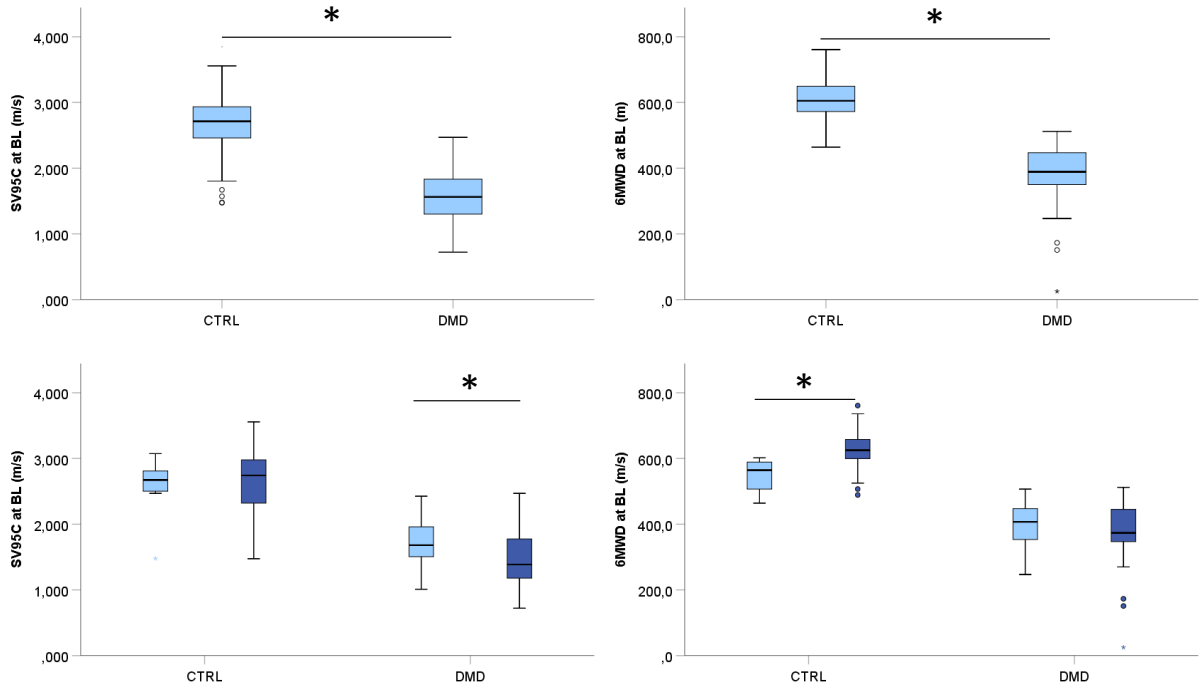
4SC = 4-stair climb test; 6MWD = 6-minute walking distance; CTRL = control population; DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

*Independent-Samples Mann-Whitney U Test

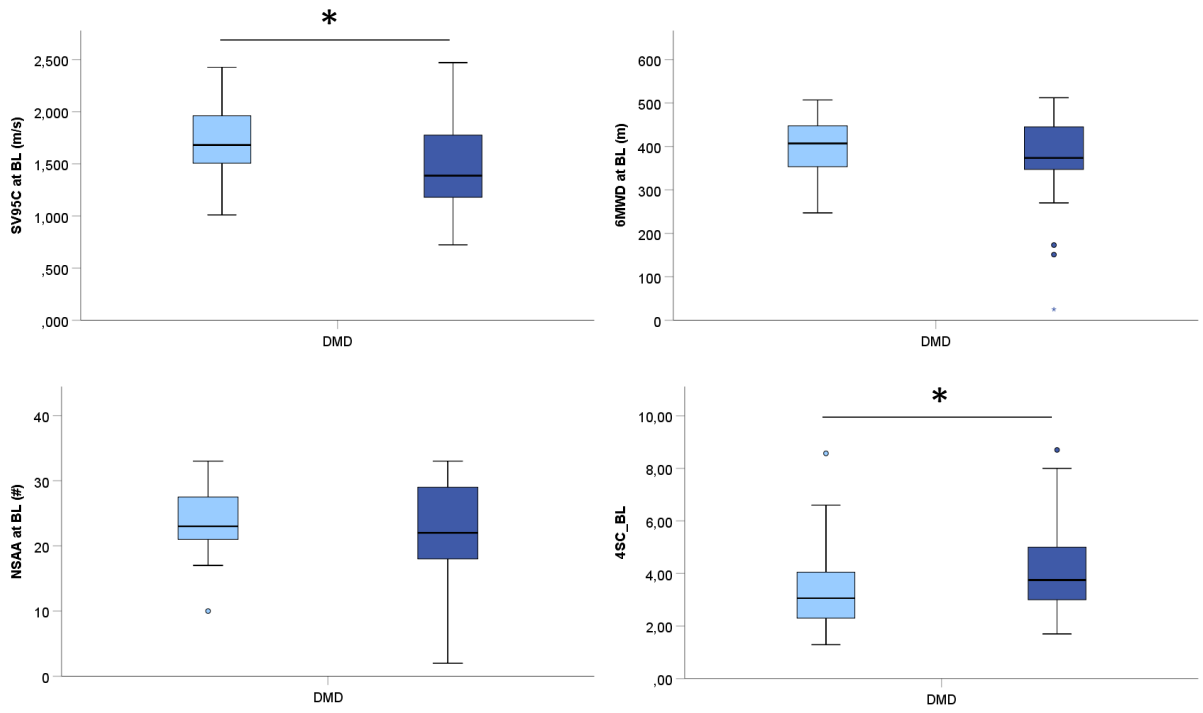
Graphical representations of the comparison between DMD patients and healthy control subjects at Baseline for SV95C and 6MWD, and the age range effect within the DMD population for SV95C, 6MWD, NSAA, and 4SC and the healthy control population for SV95C and 6MWD are presented in Figure 7.

Figure 7: Comparison of SV95C Between the DMD Patient and Healthy Control Subjects at Baseline and the Age Range Effect in the DMD and Healthy Control Populations

A. Comparison between DMD and CTRL subjects at baseline (SV95C and 6MWD)



B. Age range effect – DMD population (SV95C, 6MWD, NSAA, and 4SC)



[5-7] [8-14]

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; CTRL = Control population, DMD = Duchenne muscular dystrophy, NSAA = North Star Ambulatory Assessment; SD = standard deviation;

SV95C = 95th centile of the stride velocity

* Change statistically significant (one-sample Wilcoxon signed rank test)

3.2.2.3.2. Convergent Validity

Baseline characteristics including age and mean/median scores of the SV95C, 6MWD, 4SC, and NSAA (total score) for the DMD population (N = 107) are summarized in Table 32.

Table 32: Baseline Characteristics Including Age, SV95C and Existing COA Scores at Baseline

	N	Mean	Median	SD	Min	Max
Age (y)	107	8.3	8.0	1.9	5.0	14.0
SV95C (m/s)	107	1.586	1.576	0.379	0.723	2.470
6MWD (m)	107	390.0	389.0	75.7	25.0	512.0
NSAA (#)	107	22.9	23.0	6.3	2	33
4SC (s)	107	3.82	3.40	1.58	1.29	8.70

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; COA = clinical outcome assessment; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

Correlation coefficients between SV95C and the existing COAs (6MWD, 4SC, and NSAA) at Baseline in 107 DMD patients are presented in Table 33. Overall, SV95C was significantly correlated with the 6MWD, NSAA and 4SC (P-values < 0.001), with correlation coefficients (parametric [Pearson] and non-parametric [Spearman's]) ranging from -0.634 to 0.678. Specifically, there is a good correlation between the 6MWD and SV95C, mostly for SV95C below 1.5 m/s. For patients with SV95C > 1.5 m/s, the correlation is not as good, likely due to ceiling effect of the 6MWD around 500 m related to the test instruction asking patients to walk as fast as possible but not to run which does not exist for SV95C suggesting that SV95C is more able to discriminate than 6MWD. These results are displayed graphically by age group (5 to 7 years of age and 8 to 14 years of age) in Figure 8.

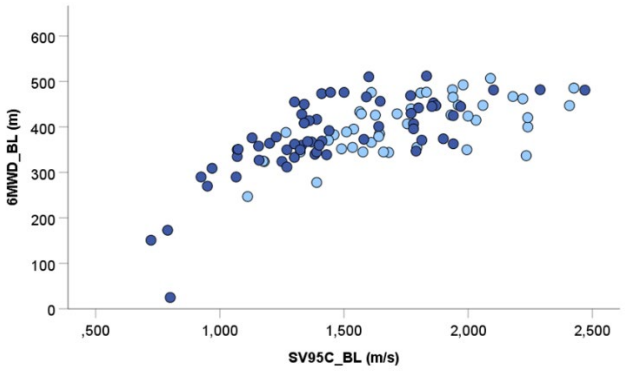
Table 33: Correlation Matrix Between SV95C and Other Functioning Outcome Measures at Baseline

	Non-parametric correlation	**SV95C_BL**	**6MWD_BL**	**NSAA_BL**	**4SC_BL**	***Parametric correlation***
SV95C_BL			*0.678*	*0.676*	*-0.622*	*Pearson*
			<0.001	*<0.001*	*<0.001*	*Sig. (bilat)*
			107	*107*	*107*	*N*
6MWD_BL	Spearman's Rho	0.657		*0.746*	*-0.531*	*Pearson*
	Sig. (bilat)	<0.001		*<0.001*	*<0.001*	*Sig. (bilat)*
	N	107		*107*	*107*	*N*
NSAA_BL	Spearman's Rho	0.644	0.674		*-0.641*	*Pearson*
	Sig. (bilat)	<0.001	<0.001		*<0.001*	*Sig. (bilat)*
	N	107	107		*107*	*N*
4SC_BL	Spearman's Rho	-0.634	-0.518	-0.639		
	Sig. (bilat)	<0.001	<0.001	<0.001		
	N	107	107	107		

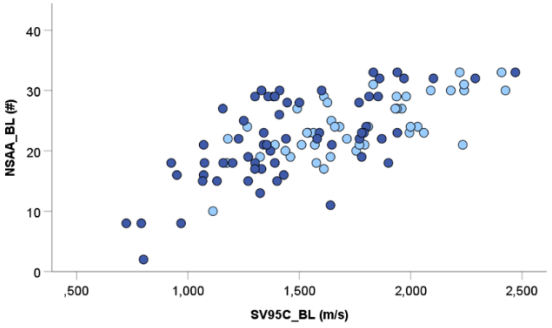
4SC = 4-stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

Figure 8: Relationship Between SV95C and Other Functioning Outcome Measures at Baseline (by Age Group)

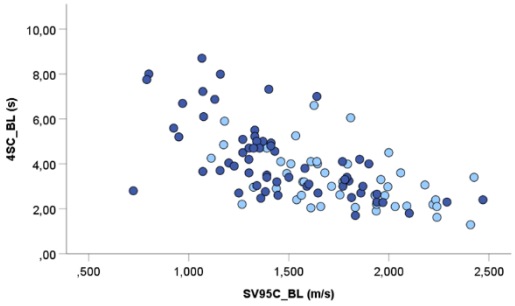
A (6MWD)



B (NSAA)



C (4SC)



[5-7] [8-14]

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; NSAA = North star ambulatory assessment; SV95C = 95th centile of the stride velocity

Population characteristics of patients followed over 3 months, including age and mean/median scores of the SV95C, 6MWD, 4SC, and NSAA (total score) for the DMD population (N = 43) at Baseline are summarized in Table 34.

Table 34: Population Characteristics Including Age, SV95C and Existing COA Scores at Baseline

Baseline Values	N	Mean	Median	SD	Min	Max
Age (y)	43	8.395	8.000	1.5757	6.0	11.0
SV95C (m/s)	43	1.576	1.590	0.343	0.790	2.240
6MWD (m)	43	387.1	383.0	60.2	173.0	507.0
NSAA (#)	43	22.1	22.0	6.0	8	33
4SC (s)	43	3.90	3.50	1.50	1.62	7.75

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; COA = clinical outcome assessment; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

Table 35 presents correlation coefficients between SV95C and the existing COAs (6MWD, 4SC, and NSAA) at Month 3 and the change after 3 months of follow-up in 43 DMD patients. Overall, the SV95C was significantly correlated with 6MWD, NSAA and 4SC at Month 3 (Pearson and Spearman's correlation coefficients ranged from -0.603 to 0.761; P-values < 0.001) but was not correlated with other COAs based on the change after 3 months of follow-up (correlations ranged from -0.281 to -0.009; P-values > 0.05). This was not unexpected as only the SV95C significantly decreased after 3 months of follow-up (see Section 3.2.2.4). These results are also displayed graphically by age group (5 to 7 years of age and 8 to 14 years of age) in Figure 9.

Table 35: Correlation Matrix Between SV95C Changes at 3 Months and Other Functioning Outcome Measures

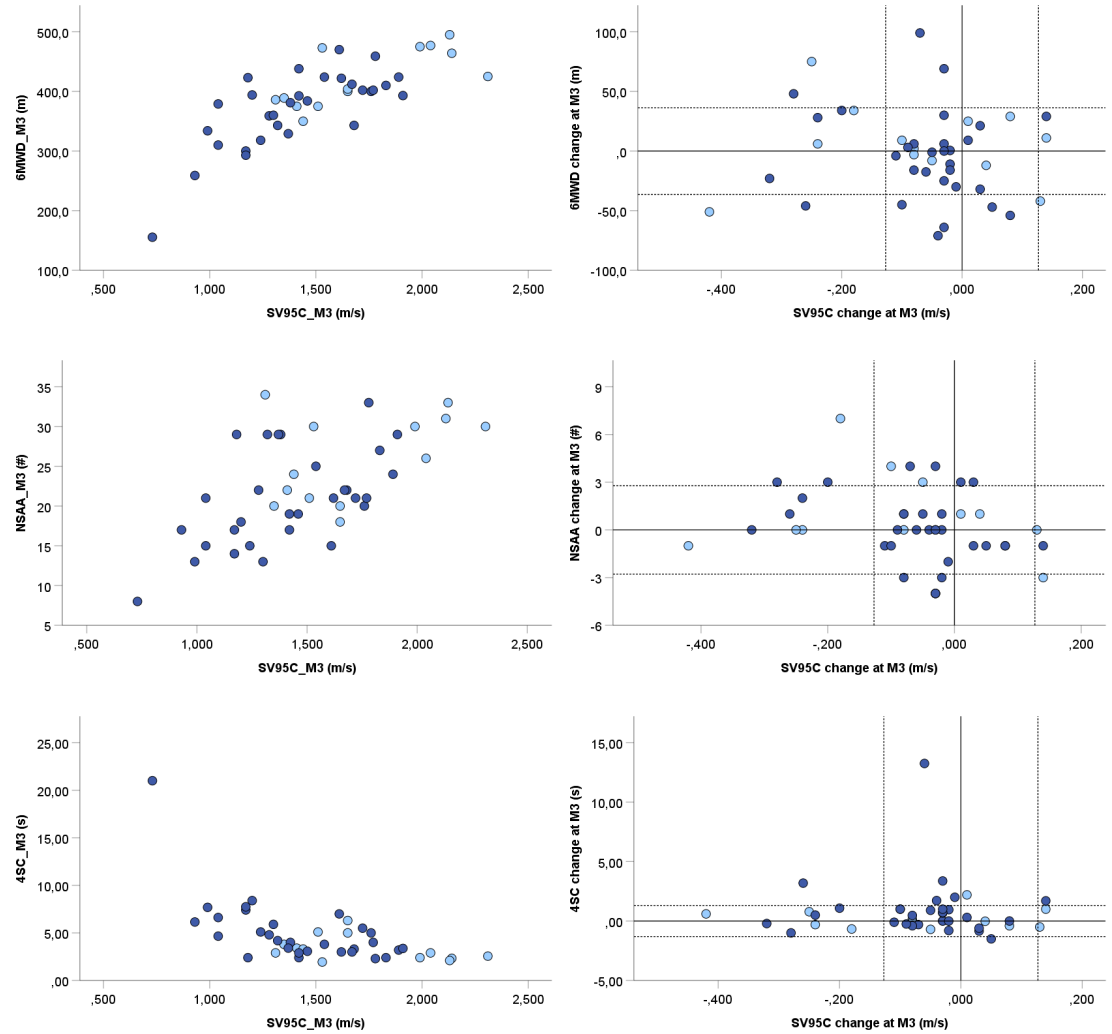
	Non-parametric Correlation	SV95C_M3	6MWD_M3	NSAA_M3	4SC_M3	SV95C_d3M	6MWD_d3M	NSAA_d3M	4SC_d3M	*Parametric correlation*
SV95C_M3			*0.761***	*0.599***	*-0.587***	*0.233*	*0.115*	*-0.052*	*-0.382**	*Pearson*
			<0.001	*<0.001*	*<0.001*	*0.132*	*0.462*	*0.742*	*0.012*	Sig. (bilat)
			43	*43*	*43*	*43*	*43*	*43*	*43*	*N*
6MWD_M3	Spearman's Rho	0.761**		*0.573***	*-0.712***	*0.072*	*0.398***	*0.162*	*-0.643***	*Pearson*
	Sig. (bilat)	<0.001		*<0.001*	*<0.001*	*0.645*	*0.008*	*0.300*	*<0.001*	Sig. (bilat)
	N	43		*43*	*43*	*43*	*43*	*43*	*43*	*N*
NSAA_M3	Spearman's Rho	*0.*575**	0.500**		*-0.684***	*0.037*	*0.121*	*0.301**	*-0.472***	*Pearson*
	Sig. (bilat)	<0.001	0.001		*<0.001*	*0.812*	*0.438*	*0.050*	*0.001*	Sig. (bilat)
	N	43	43		*43*	*43*	*43*	*43*	*43*	*N*
4SC_M3	Spearman's Rho	-0.603**	-0.641**	-0.771**		*-0.003*	*-0.109*	*-0.081*	*0.893***	*Pearson*
	Sig. (bilat)	<0.001	<0.001	<0.001		*0.985*	*0.487*	*0.607*	*<0.001*	Sig. (bilat)
	N	43	43	43		*43*	*43*	*43*	*43*	*N*
SV95C_d3M	Spearman's Rho	0.229	0.154	0.025	-0.019		*-0.093*	*-0.254*	*-0.009*	*Pearson*
	Sig. (bilat)	0.140	0.325	0.874	0.906		*0.552*	*0.100*	*0.955*	Sig. (bilat)
	N	43	43	43	43		*43*	*43*	*43*	*N*
6MWD_d3M	Spearman's Rho	0.157	0.410**	0.103	-0.115	-0.104		*0.398*	*-0.177*	*Pearson*
	Sig. (bilat)	0.314	0.006	0.512	0.463	0.509		*0.008*	*0.256*	Sig. (bilat)

	Non-parametric Correlation	**SV95C_M3**	**6MWD_M3**	**NSAA_M3**	**4SC_M3**	**SV95C_d3M**	**6MWD_d3M**	**NSAA_d3M**	**4SC_d3M**	***Parametric correlation***
	N	43	43	43	43	43		*43*	*43*	*N*
NSAA_d3M	Spearman's Rho	-0.067	0.174	0.287	-0.121	-0.281	0.352*		*-0.143*	*Pearson*
	Sig. (bilat)	0.667	0.264	0.062	0.438	0.068	0.020		*0.359*	Sig. (bilat)
	N	43	43	43	43	43	43		*43*	*N*
4SC_d3M	Spearman's Rho	-0.203	-0.339*	-0.395**	0.658**	-0.045	-0.161	-0.191		
	Sig. (bilat)	0.191	0.026	0.009	<0.001	0.773	0.301	0.221		
	N	43	43	43	43	43	43	43		

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

*Correlation is significant at the 0.01 level (2-tailed); **Correlation is significant at the 0.05 level (2-tailed); d3M : change after 3 months of FU

Figure 9: Relationship Between SV95C and Other Functional Outcome Measures at 3 Months (by Age Group)



4SC = 4-stair climb test; 6MWD = 6-minute walking distance; MDC = Minimal detectable change at 80% of confidence level; M3 = Months 3; NSAA = North star ambulatory assessment; SV95C = 95th centile of the stride velocity

Population characteristics of patients followed over 6 months, including age and mean/median (SD) [min-max] scores of the SV95C, 6MWD, 4SC, and NSAA (total score) for the DMD population (N = 20) at Baseline are summarized in Table 36.

Table 36: Population Characteristics Including Age, SV95C and Existing COA Scores at Baseline

Baseline Values	N	Mean	Median	SD	Min	Max
Age (y)	20	8.5	8.5	1.8	6.0	11.0
SV95C (m/s)	20	1.654	1.640	0.257	1.130	2.240
6MWD (m)	20	402.8	398.0	47.1	312.0	510.3

Baseline Values	N	Mean	Median	SD	Min	Max
NSAA (#)	20	21.75	22.00	5.533	11	32
4SC (s)	20	3.7510	3.4500	1.32003	1.62	7.00

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; COA = clinical outcome assessment; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

Correlation coefficients between the SV95C and the existing COAs (6MWD, 4SC, and NSAA) at Month 6 and the change after 6 months of follow-up in 20 DMD patients are presented in Table 37. Overall, the SV95C was significantly (or nearly significantly) correlated with the 6MWD, NSAA, and 4SC at Month 6 (Pearson and Spearman's correlations ranged from -0.536 to 0.547; P-values ranged from 0.013 to 0.084) but was not correlated with other COAs based on the change after 6 months of follow-up (correlations ranged from -0.256 to 0.014; P-values > 0.05). This was not unexpected as only the SV95C significantly decreased after 6 months of follow-up (see Section 3.2.2.4). These results are also displayed graphically by age group (5 to 7 years of age and 8 to 14 years of age) in Figure 10.

Table 37: Correlation Matrix Between SV95C Changes at 6 Months and Other Functioning Outcome Measures at Month 6

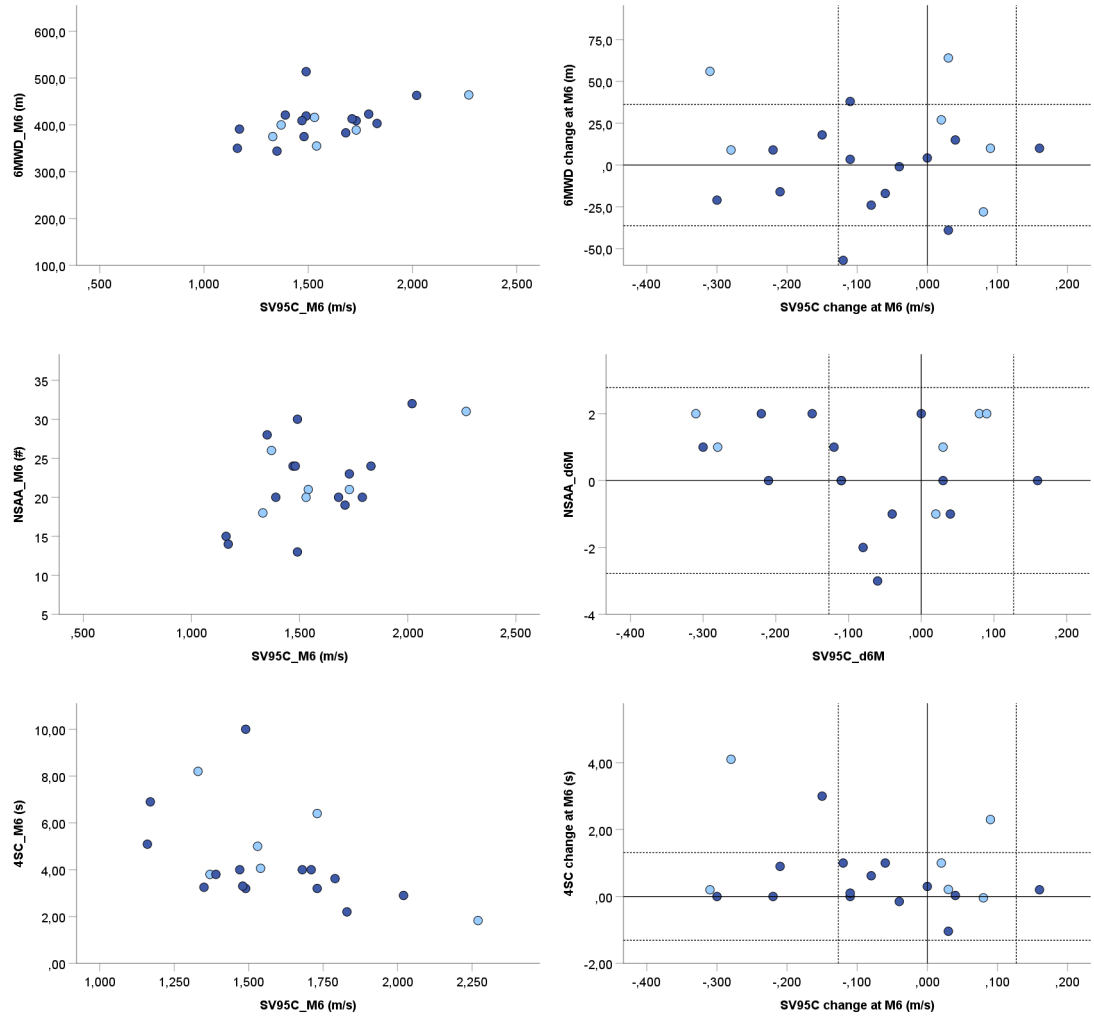
	Non-parametric correlation	**SV95C_M6**	**6MWD_M6**	**NSAA_M6**	**4SC_M6**	**SV95C_d6M**	**6MWD_d6M**	**NSAA_d6M**	**4SC_d6M**	***Parametric correlation***
SV95C_M6			*0.507**	*0.547**	*-0.486**	*0.372*	*0.008*	*-0.055*	*-0.125*	*Pearson*
			0.022	*0.013*	*0.030*	*0.106*	*0.972*	*0.817*	*0.600*	Sig. (bilat)
			20	*20*	*20*	*20*	*20*	*20*	*20*	*N*
6MWD_M6	Spearman's Rho	0.524*		*0.487**	*-0.255*	*0.202*	*0.145*	*-0.110*	*-0.042*	*Pearson*
	Sig. (bilat)	0.018		*0.029*	*0.278*	*0.393*	*0.541*	*0.644*	*0.859*	Sig. (bilat)
	N	20		*20*	*20*	*20*	*20*	*20*	*20*	*N*
NSAA_M6	Spearman's Rho	0.396	0.244		*-0.748***	*0.161*	*0.037*	*0.019*	*-0.405*	*Pearson*
	Sig. (bilat)	0.084	0.299		*<0.001*	*0.498*	*0.876*	*0.937*	*0.076*	Sig. (bilat)
	N	20	20		*20*	*20*	*20*	*20*	*20*	*N*
4SC_M6	Spearman's Rho	-0.536*	-0.393	-0.824**		*-0.166*	*0.169*	*0.205*	*0.782***	*Pearson*
	Sig. (bilat)	0.015	0.086	<0.001		*0.485*	*0.475*	*0.386*	*<0.001*	Sig. (bilat)
	N	20	20	20		*20*	*20*	*20*	*20*	*N*
SV95C_d6M	Spearman's Rho	0.401	0.184	0.138	-0.106		*-0.036*	*-0.198*	*-0.256*	*Pearson*
	Sig. (bilat)	0.080	0.436	0.561	0.657		*0.880*	*0.402*	*0.277*	Sig. (bilat)
	N	20	20	20	20		*20*	*20*	*20*	*N*
6MWD_d6M	Spearman's Rho	-0.175	0.114	-0.135	0.252	0.014		*0.199*	*0.097*	*Pearson*
	Sig. (bilat)	0.460	0.632	0.571	0.284	0.952		*0.400*	*0.684*	Sig. (bilat)
	N	20	20	20	20	20		*20*	*20*	*N*

	Non-parametric correlation	**SV95C_M6**	**6MWD_M6**	**NSAA_M6**	**4SC_M6**	**SV95C_d6M**	**6MWD_d6M**	**NSAA_d6M**	**4SC_d6M**	***Parametric correlation***
NSAA_d6M	Spearman's Rho	-0.066	-0.155	0.034	0.219	-0.205	0.172		*0.187*	*Pearson*
	Sig. (bilat)	0.783	0.514	0.886	0.354	0.385	0.469		*0.431*	Sig. (bilat)
	N	20	20	20	20	20	20		*20*	*N*
4SC_d6M	Spearman's Rho	0.070	0.379	-0.344	0.437	-0.155	0.198	0.079		
	Sig. (bilat)	0.769	0.099	0.138	0.054	0.513	0.402	0.742		
	N	20	20	20	20	20	20	20		

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

**Correlation is significant at the 0.01 level (2-tailed);*Correlation is significant at the 0.05 level (2-tailed); d6M : change after 6 months of FU

Figure 10: Relationship Between SV95C and Other Functional Outcome Measures at 6 Months (by Age Group)



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------ : ± MDC80%

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; MDC80% = Minimal detectable change at 80% of confidence level; M6 = Months 6; NSAA = North star ambulatory assessment; SV95C = 95th centile of the stride velocity

Population characteristics of patients followed over 9 months, including age and mean/median scores of the SV95C, 6MWD, 4SC, and NSAA (total score) for the DMD population (N = 24) at Baseline are summarized in Table 38.

Table 38: Population Characteristics Including Age, SV95C and Existing COA Scores at Baseline

BL Values	N	Mean	Median	SD	Min	Max
Age (y)	24	8.3	8.0	1.6	6.0	11.0
SV95C (m/s)	24	1.663	1.705	0.362	0.950	2.240
6MWD (m)	24	409.3	407.0	55.4	270.0	510.3

BL Values	N	Mean	Median	SD	Min	Max
NSAA (#)	24	23.75	22.50	5.64	16	33
4SC (s)	24	3.42	3.20	1.20	1.62	7.22

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; COA = clinical outcome assessment; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

Table 39 presents correlation coefficients between the SV95C and the existing COAs (6MWD, 4SC, and NSAA) at Month 9 and the change after 9 months of follow-up in 24 DMD patients. Overall, the SV95C was significantly correlated with the 6MWD, NSAA, and 4SC at Month 9 (Pearson and Spearman's correlations ranged from -0.651 to 0.698; P-values ranged from < 0.001 to 0.001) but was not correlated with other COAs based on the change after 9 months of follow-up despite changes also observed in existing COAs (correlations ranged from -0.368 to 0.094; P-values > 0.05). These results are also displayed graphically by age group (5 to 7 years of age and 8 to 14 years of age) in Figure 11.

Table 39: Correlation Matrix Between SV95C Changes at 9 Months and Other Functioning Outcome Measures

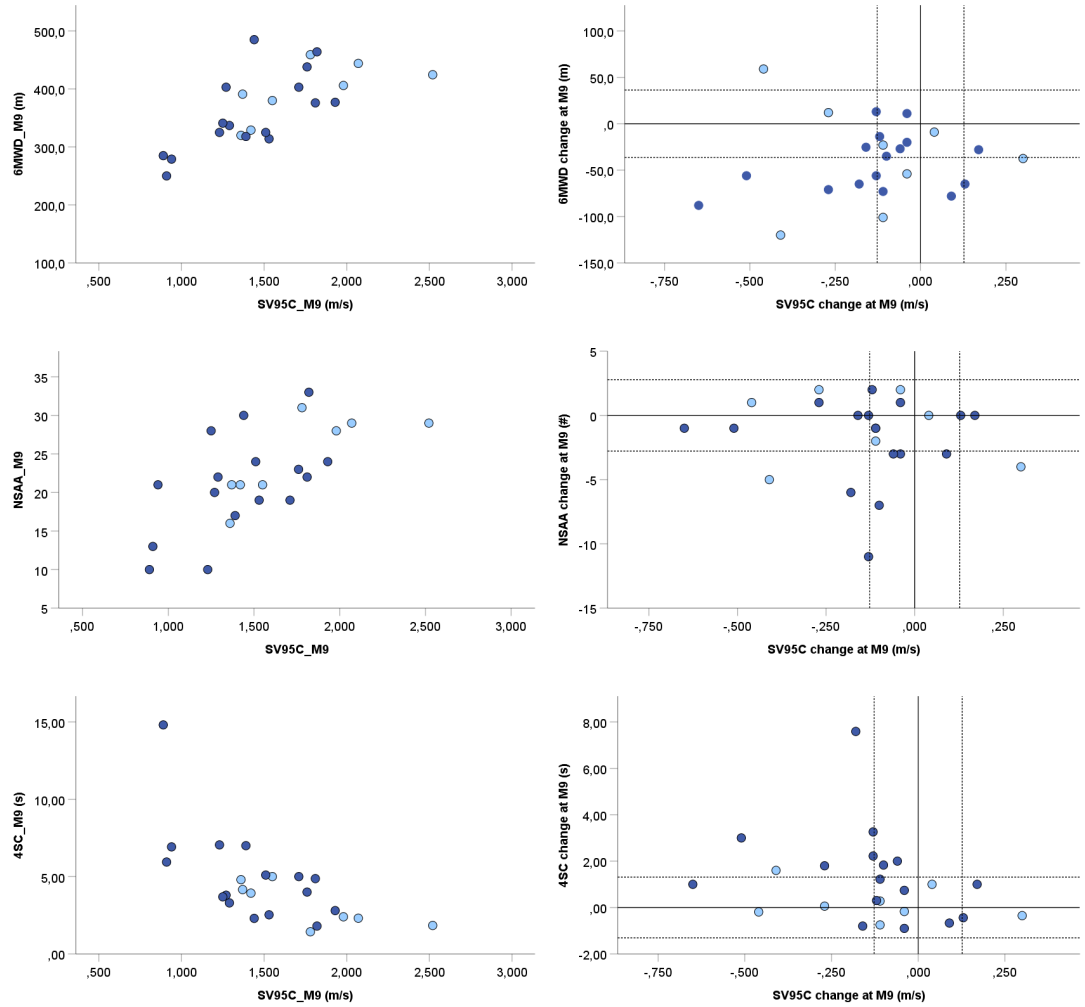
	Non-parametric Correlation	**SV95C_M9**	**6MWD_M9**	**NSAA_M9**	**4SC_M9**	**SV95C_d9M**	**6MWD_d9M**	**NSAA_d9M**	**4SC_d9M**	***Parametric Correlation***
SV95C_M9			*0.698***	*0.658***	*-0.647***	*0.400*	*0.186*	*-0.008*	*-0.545***	*Pearson*
			<0.001	*<0.001*	*0.001*	*0.053*	*0.385*	*0.970*	*0.006*	Sig. (bilat)
			24	*24*	*24*	*24*	*24*	*24*	*24*	*N*
6MWD_M9	Spearman's Rho	0.691**		*0.764***	*-0.636***	*0.180*	*0.510**	*0.288*	*-0.571***	*Pearson*
	Sig. (bilat)	<0.001		*<0.001*	*0.001*	*0.399*	*0.011*	*0.173*	*0.004*	Sig. (bilat)
	N	24		*24*	*24*	*24*	*24*	*24*	*24*	*N*
NSAA_M9	Spearman's Rho	0.677**	0.769**		*-0.771***	*0.118*	*0.276*	*0.440**	*-0.638***	*Pearson*
	Sig. (bilat)	<0.001	<0.001		*<0.001*	*0.582*	*0.191*	*0.031*	*0.001*	Sig. (bilat)
	N	24	24		*24*	*24*	*24*	*24*	*24*	*N*
4SC_M9	Spearman's Rho	-0.651**	-0.739**	-0.810**		*-0.154*	*-0.198*	*-0.390*	*0.943***	*Pearson*
	Sig. (bilat)	0.001	<0.001	<0.001		*0.474*	*0.353*	*0.060*	*<0.001*	Sig. (bilat)
	N	24	24	24		*24*	*24*	*24*	*24*	*N*
SV95C_d9M	Spearman's Rho	0.380	0.164	0.076	-0.189		*0.091*	*-0.068*	*-0.256*	*Pearson*
	Sig. (bilat)	0.067	0.444	0.724	0.377		*0.674*	*0.752*	*0.227*	Sig. (bilat)

	Non-parametric Correlation	**SV95C_M9**	**6MWD_M9**	**NSAA_M9**	**4SC_M9**	**SV95C_d9M**	**6MWD_d9M**	**NSAA_d9M**	**4SC_d9M**	***Parametric Correlation***
	N	24	24	24	24		*24*	*24*	*24*	*N*
6MWD_d9M	Spearman's Rho	0.200	0.475*	0.204	-0.117	0.094		*0.134*	*-0.241*	*Pearson*
	Sig. (bilat)	0.350	0.019	0.339	0.587	0.662		*0.534*	*0.257*	Sig. (bilat)
	N	24	24	24	24	24		*24*	*24*	*N*
NSAA_d9M	Spearman's Rho	0.068	0.358	0.404	-0.298	-0.086	0.305		*-0.440**	*Pearson*
	Sig. (bilat)	0.752	0.086	0.051	0.157	0.691	0.147		*0.031*	Sig. (bilat)
	N	24	24	24	24	24	24		*24*	*N*
4SC_d9M	Spearman's Rho	-0.542**	-0.614**	-0.615**	0.859**	-0.368	-0.227	-0.399		
	Sig. (bilat)	0.006	0.001	0.001	<0.001	0.077	0.286	0.053		
	N	24	24	24	24	24	24	24		

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

**Correlation is significant at the 0.01 level (2-tailed);*Correlation is significant at the 0.05 level (2-tailed); d9M : change after 9 months of FU

Figure 11: Relationship Between SV95C and Other Functional Outcome Measures at 9 Months (by Age Group)



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------ : ± MDC80%

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; MDC80% = minimal detectable change at 80% of confidence level; M9 = Months 9; NSAA = North star ambulatory assessment; SV95C = 95th centile of the stride velocity

Population characteristics of patients followed over 12 months, including age and mean/median (SD) [min-max] scores of the SV95C, 6MWD, 4SC, and NSAA (total score) for the DMD population (N = 15) at Baseline are summarized in Table 40.

Table 40: Population Characteristics Including Age, SV95C and Existing COA Scores at Baseline

Baseline Values	N	Mean	Median	SD	Min	Max
Age (y)	15	8.333	8.000	1.5430	6.0	11.0
SV95C (m/s)	15	1.59600	1.64000	0.403977	0.950	2.240
6MWD (m)	15	380.333	383.000	52.0380	270.0	462.0
NSAA (#)	15	21.93	21.00	5.325	15	33
4SC (s)	15	3.7213	3.4000	1.37595	1.62	7.22

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; COA = clinical outcome assessment; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SV95C = 95th centile of the stride velocity

Table 41 presents correlation coefficients between the SV95C and the existing COAs (6MWD, 4SC, and NSAA) at Month 12 and the change after 12 months of follow-up in 15 DMD patients. Overall, the SV95C was significantly correlated with the 6MWD, NSAA, and 4SC at Month 12 (Pearson and Spearman's correlations ranged from -0.749 to 0.835; P-values ranged from < 0.001 to 0.013). Based on the change after 12 months of follow-up, the SV95C appeared to be more correlated with the other COAs, although these correlations were not statistically significant (with the exception of the correlation between SV95C and 4SC; correlations ranged from -0.593 to 0.499). These results are also displayed graphically by age group (5 to 7 years of age and 8 to 14 years of age) in Figure 12.

Overall, correlations observed between SV95C and existing COAs at baseline and over time for 12 months follow up were as expected meaning that a relationship was found between all variables. As all variables aim to characterize patient ambulatory capabilities, they are linked through the patient clinical status as highlighted by the relationship with NSAA score that represents a more global evaluation of the motor abilities of a patient not limited to the walking ability such as timed tests. Nevertheless, a correlation between 2 variables does not imply a cause/consequence relationship. Consequently, a strong correlation between variable changes was not expected because their evolution with time, their variability, and their ability to detect patient ambulation decline are different (see Section 3.2.2.4).

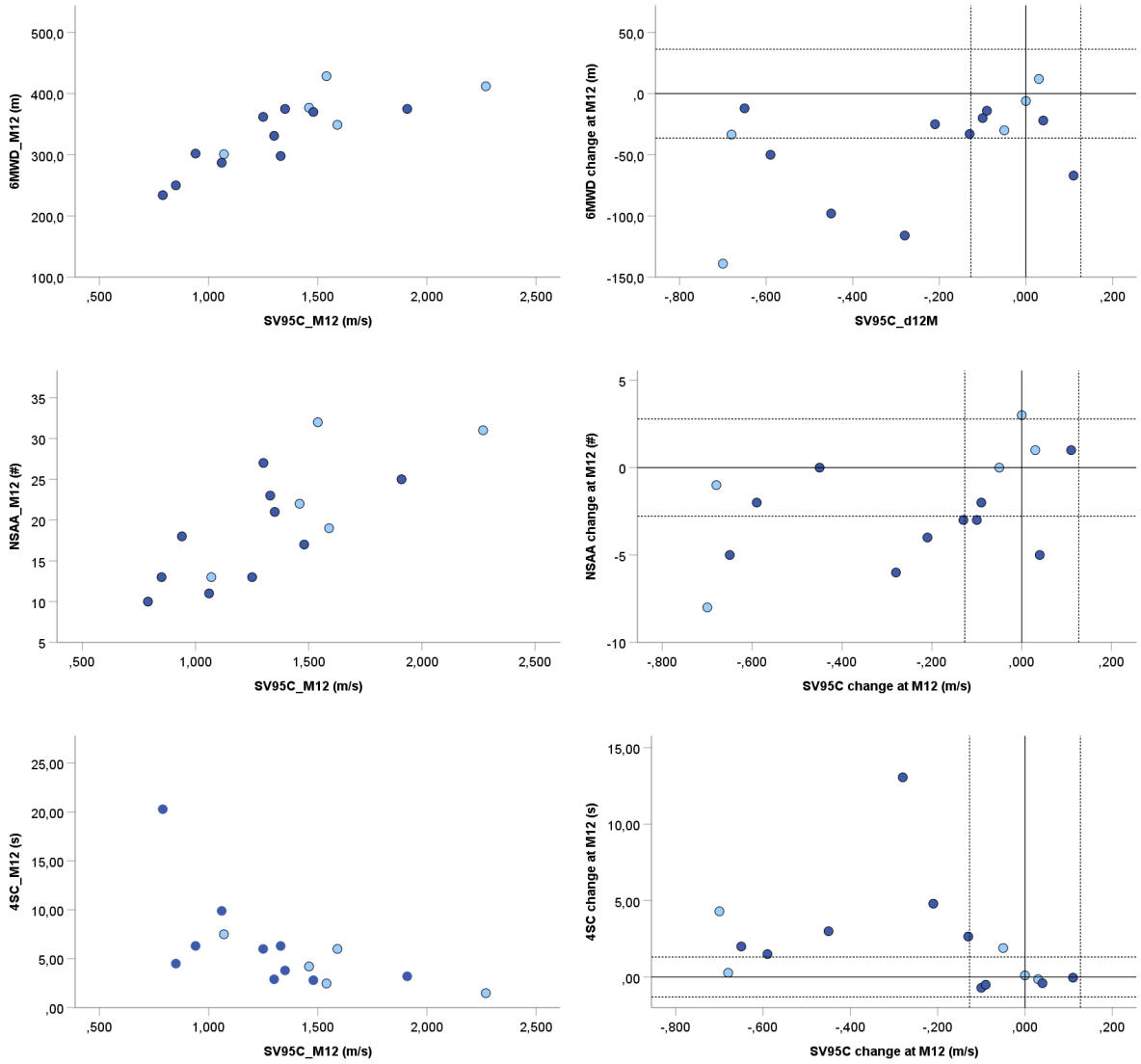
Table 41: Correlation Matrix Between SV95C Changes at 12 Months and Other Functioning Outcome Measures

	Non-parametric Correlation	**SV95C_M12**	**6MWD_M12**	**NSAA_M12**	**4SC_M12**	**SV95C_d12M**	**6MWD_d12M**	**NSAA_d12M**	**4SC_d12M**	***Parametric Correlation***
SV95C_M12			*0.821***	*0.758***	*-0.625**	*0.335*	*0.387*	*0.655***	*-0.539**	*Pearson*
			<0.001	*0.001*	*0.013*	*0.223*	*0.154*	*0.008*	*0.038*	Sig. (bilat)
			15	*15*	*15*	*15*	*15*	*15*	*15*	*N*
6MWD_M12	Spearman's Rho	0.835**		*0.750***	*-0.723***	*0.006*	*0.485*	*0.523**	*-0.627**	*Pearson*
	Sig. (bilat)	<0.001		*0.001*	*0.002*	*0.984*	*0.067*	*0.045*	*0.012*	Sig. (bilat)
	N	15		*15*	*15*	*15*	*15*	*15*	*15*	*N*
NSAA_M12	Spearman's Rho	0.753**	0.734**		*-0.672***	*0.138*	*0.345*	*0.713***	*-0.579**	*Pearson*
	Sig. (bilat)	0.001	0.002		*0.006*	*0.623*	*0.207*	*0.003*	*0.024*	Sig. (bilat)
	N	15	15		*15*	*15*	*15*	*15*	*15*	*N*
4SC_M12	Spearman's Rho	-0.749**	-0.805**	-0.775**		*-0.167*	*-0.576**	*-0.507*	*0.976***	*Pearson*
	Sig. (bilat)	0.001	<0.001	0.001		*0.552*	*0.025*	*0.054*	*<0.001*	Sig. (bilat)
	N	15	15	15		*15*	*15*	*15*	*15*	*N*
SV95C_d12M	Spearman's Rho	0.468	0.248	0.262	-0.433		*0.443*	*0.456*	*-0.269*	*Pearson*
	Sig. (bilat)	0.079	0.372	0.346	0.107		*0.099*	*0.088*	*0.333*	Sig. (bilat)

	Non-parametric Correlation	SV95C_M12	6MWD_M12	NSAA_M12	4SC_M12	SV95C_d12M	6MWD_d12M	NSAA_d12M	4SC_d12M	*Parametric Correlation*
	N	15	15	15	15		*15*	*15*	*15*	*N*
6MWD_d12M	Spearman's Rho	0.229	0.359	0.197	-0.477	0.457		*0.464*	*-0.645***	*Pearson*
	Sig. (bilat)	0.413	0.188	0.481	0.072	0.087		*0.081*	*0.009*	Sig. (bilat)
	N	15	15	15	15	15		*15*	*15*	*N*
NSAA_d12M	Spearman's Rho	0.710**	0.592*	0.788**	-0.526*	0.499	0.305		*-0.514*	*Pearson*
	Sig. (bilat)	0.003	0.020	<0.001	0.044	0.058	0.269		*0.050*	Sig. (bilat)
	N	15	15	15	15	15	15		*15*	*N*
4SC_d12M	Spearman's Rho	-0.439	-0.456	-0.513	0.831**	-0.593*	-0.589*	-0.414		
	Sig. (bilat)	0.101	0.088	0.051	<0.001	0.020	0.021	0.125		
	N	15	15	15	15	15	15	15		

4SC = 4-stair climb test; 6MWD = 6-minute walking distance; NSAA = North Star Ambulatory Assessment**Correlation is significant at the 0.01 level (2-tailed);*Correlation is significant at the 0.05 level (2-tailed); d12M : change after 12 months of FU

Figure 12: Relationship Between SV95C and Other Functional Outcome Measures at 12 Months (by Age Group)



[5-7] [8-14]

------ : ± MDC80%

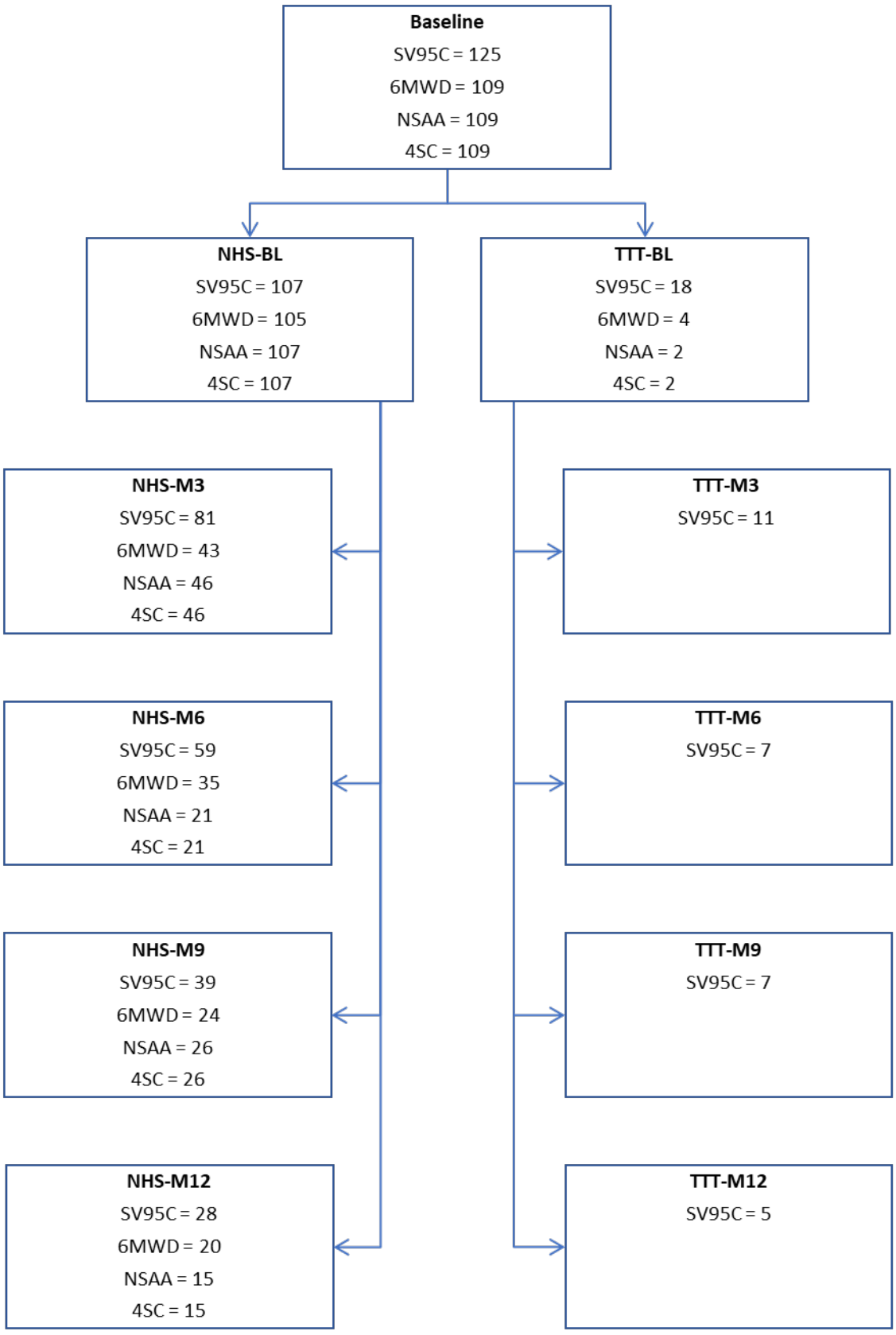
4SC = 4-stair climb test; 6MWD = 6-minute walking distance; MDC80% = minimal detectable change at 80% of confidence level; M12 = Months 12; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

3.2.2.4. Responsiveness (Ability to Detect Change)

3.2.2.4.1. Population

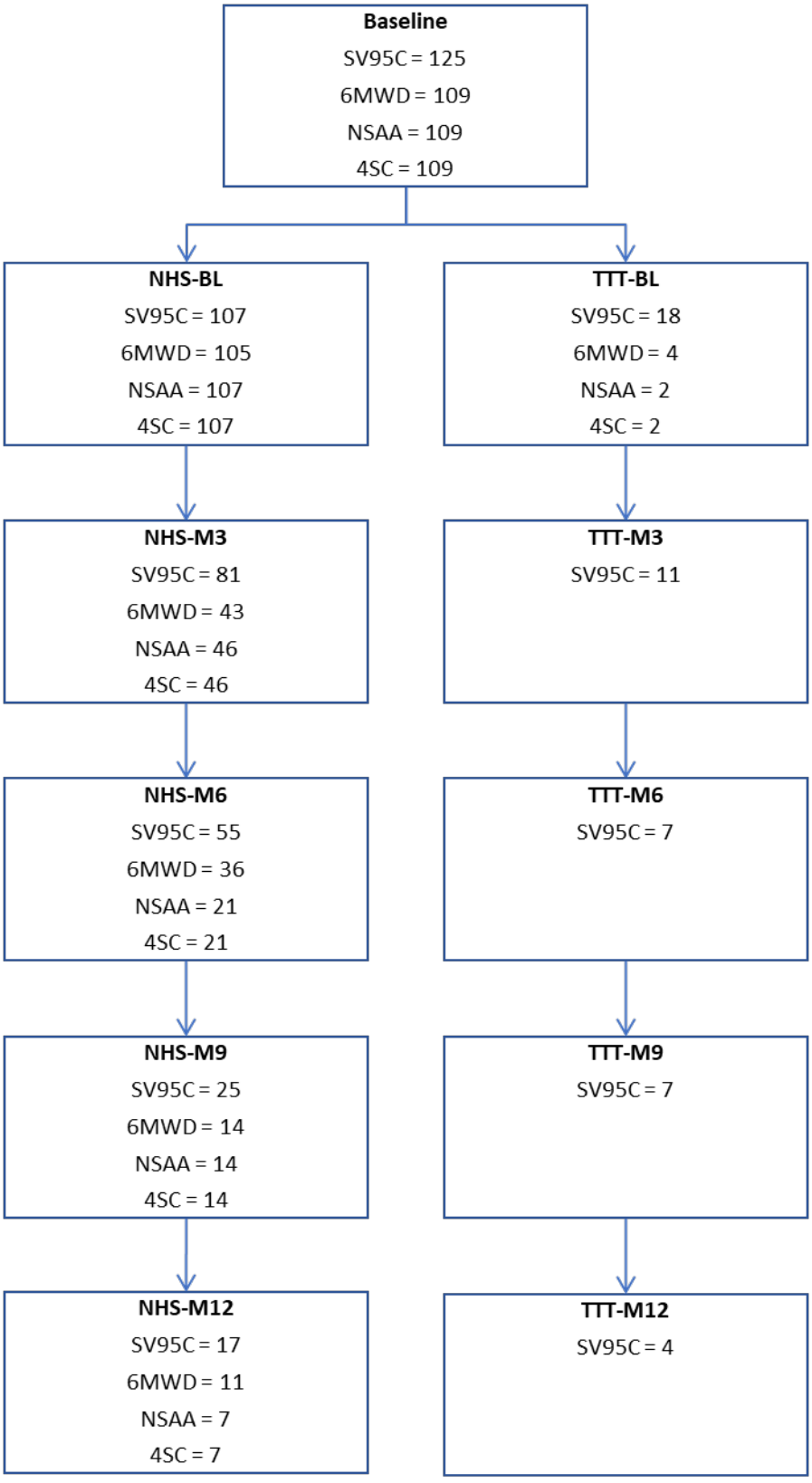
A flowchart of all available data and available data over time from the same pool of patients for the longitudinal analyses (responsiveness) is provided in Figure 13 and Figure 14, respectively.

Figure 13: Flowchart of All Available Data for Assessment of Responsiveness



4SC = 4-stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; NHS = Natural history study; NSAA = North star ambulatory assessment; SV95C = 95th centile of the stride velocity

Figure 14: Flowchart of Available Data from the Same Pool of Patients over all Time Points from BL to M12



4SC = 4-stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; NHS = Natural history study; NSAA = North Star Ambulatory Assessment; SV95C = 95th centile of the stride velocity

3.2.2.4.2. Natural Change Over Time (All Available Data)

SV95C

Responsiveness of the SV95C was determined by using the natural change over time at 3, 6, 9, and 12 months in 81, 59, 39, and 28 patients, respectively, on a stable regimen of corticosteroids or having initiated corticosteroids from at least 6 months. Overall, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.044, -0.067, -0.110, and -0.204, respectively), with statistically significant median score changes observed at each time point (P-values < 0.001). The SRM (calculated as the mean of the change divided by the SD of the change) also increased over the course of the study (from 0.45 after 3 months to 1.03 after 12 months). These results not only indicate that the loss of maximal speed is progressive over time, but that the SV95C may be sensitive enough to detect disease progression over the course of 12 months and even as early as 3 months (Table 42 and Figure 15).

Based on the estimated sample size, a large number of subjects to identify a decrease in SV95C is needed at 3 months follow-up. However, the sample size required to identify a decrease in SV95C was estimated at quite half the number after 6 months of follow-up and appeared smaller than sample size required in current clinical trials (Table 42).

Table 42: SV95C Change Over Time in Patients with DMD on Stable Steroid Regimen

SV95C Change	3M	6M	9M	12M
N	81	59	39	28
Median (m/s)	-0.044	-0.067	-0.110	-0.204
Mean (m/s)	-0.051	-0.080	-0.119	-0.241
SD (m/s)	0.113	0.134	0.186	0.234
P-value*	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
SRM**	0.45	0.60	0.64	1.03
Sample size estimated α = 5% β = 20%	78	44	38	15
Sample size estimated α = 5% β = 10%	104	58	51	20
Sample size estimated α = 5% β = 5%	128	72	63	25

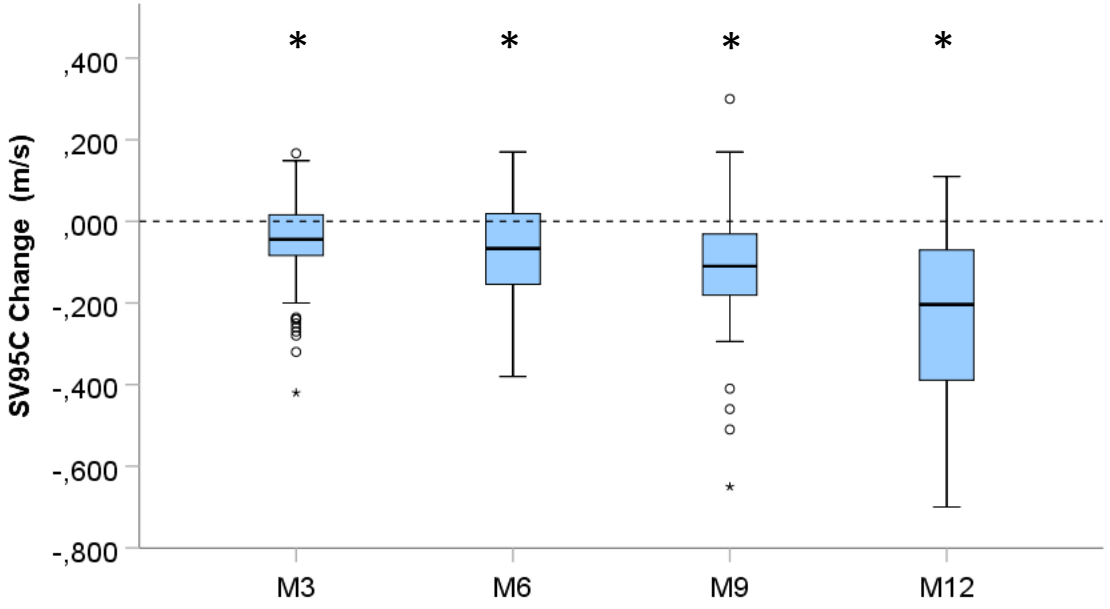
DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 15: SV95C Change Over Time in Patients with DMD



DMD = Duchenne muscular dystrophy; SV95C = 95th centile of the stride velocity

*Means that the median of the SV95C change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for each group, indicating that the loss of the maximal speed was progressive over time in each age group, with statistically significant median score changes observed at each timepoint (with the exception of the 5 to 7 year age group at Month 3; P-values < 0.05). The SRM also increased over the course of the study (from 0.66 in the 8 to 14 years of age group after 3 months to 1.01 and 1.02 in the 5 to 7 and 8 to 14 years of age group after 12 months). Of note, a larger decrease was observed in patients 8 to 14 years of age (median change from baseline scores ranged from -0.044 m/s at Month 3 to -0.210 m/s at Month 12) than patients aged 5 to 7 years (median change from baseline scores ranged from -0.023m/s at Month 3 to -0.197 m/s at Month 12). However, the difference in changes in SV95C between groups did not reach the statistical significance based on the Mann-Whitney U test (P-values > 0.05; Table 43 and Figure 16).

Furthermore, the sample size required to identify a decrease in SV95C was higher in the youngest group at 6 and 9 months of follow up but similar between age groups after 12 months of follow-up (Table 43).

Overall, these results indicate that the loss of maximal speed is progressive over time, even in youngest patients. SV95C appears sensitive enough to detect disease progression over the course of 12 months and even as early as 3 months in patients older than 8 years.

Table 43: SV95C Change Over Time in Patients with DMD on Stable Steroid Regimen (Stratified by Age Group)

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	32	49	28	31	16	23	11	17
Median (m/s)	-0.023	-0.044	-0.034	-0.084	-0.081	-0.130	-0.197	-0.210
Mean (m/s)	-0.033	-0.063	-0.071	-0.089	-0.093	-0.138	-0.263	-0.226
SD (m/s)	0.136	0.095	0.149	0.121	0.187	0.187	0.262	0.222
P-value*	0.350	**< 0.001**	**0.036**	**0.001**	**0.044**	**0.002**	**0.007**	**0.002**
SRM**	-	0.66	0.48	0.74	0.50	0.74	1.01	1.02
Sample size estimated α = 5% β = 20%	-	36	68	29	63	29	16	15
Sample size estimated α = 5% β = 10%	-	48	91	38	84	38	21	20
Sample size estimated α = 5% β = 5%	-	59	113	47	104	47	26	25
P-value***	0.179		0.430		0.437		0.746	

DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

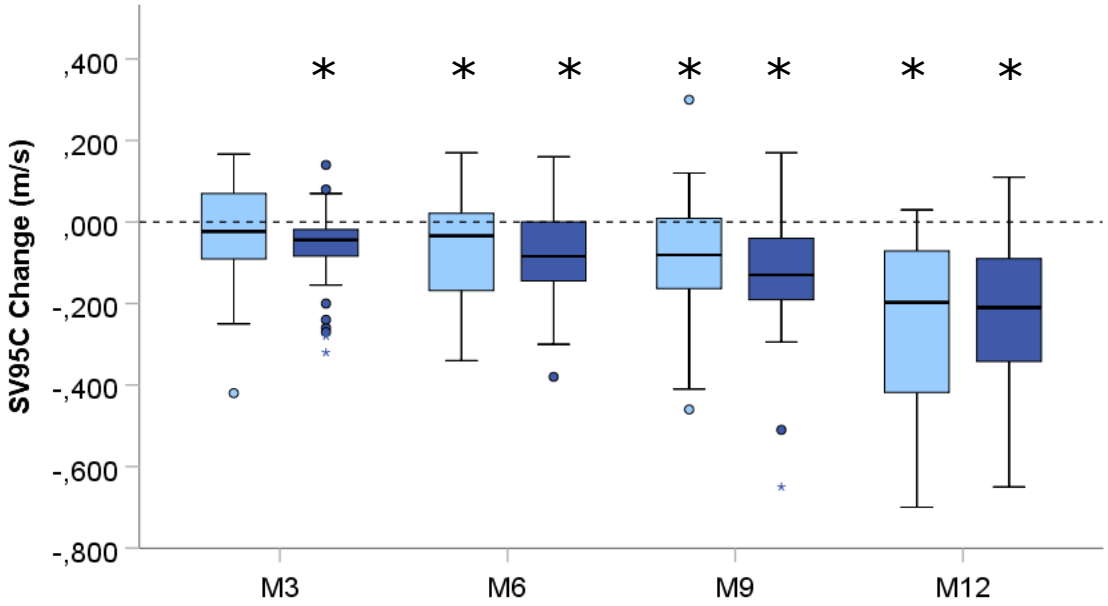
*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

*** Mann-Whitney U test to detect any age-range effect.

Figure 16: SV95C Change Over Time in Patients with DMD (Stratified by Age Groups)



DMD = Duchenne muscular dystrophy; SV95C = 95th centile of the stride velocity

*Means that the median of the SV95C change is statistically different from zero (One-sample Wilcoxon signed rank test)

6MWD

In comparison with SV95C, for the 6MWD, a decline in the median change from baseline scores was only reported after 9 months of follow-up (median change from baseline scores were 0.0 m, 3.4 m, -36.3 m, and -31.5 m at Months 3, 6, 9, and 12, respectively), with statistically significant median score changes observed at Months 9 and 12 (P-values < 0.001 and 0.003, respectively; Table 44 and
Figure 17).

Table 44: 6MWD Change Over Time in Patients with DMD

6MWD Change	3M	6M	9M	12M
N	43	35	24	20
Median (m)	0.0	3.4	-36.3	-31.5
Mean (m)	-1.0	1.1	-39.6	-37.6
SD (m)	36.7	44.4	41.0	48.2
P-value*	0.731	0.682	**< 0.001**	**0.003**
SRM**	-	-	0.97	0.78
Sample size estimated α = 5% β = 20%	-	-	17	26
Sample size estimated	-	-	23	34

6MWD Change	3M	6M	9M	12M
α = 5% β = 10%				
Sample size estimated α = 5% β = 5%	-	-	28	43

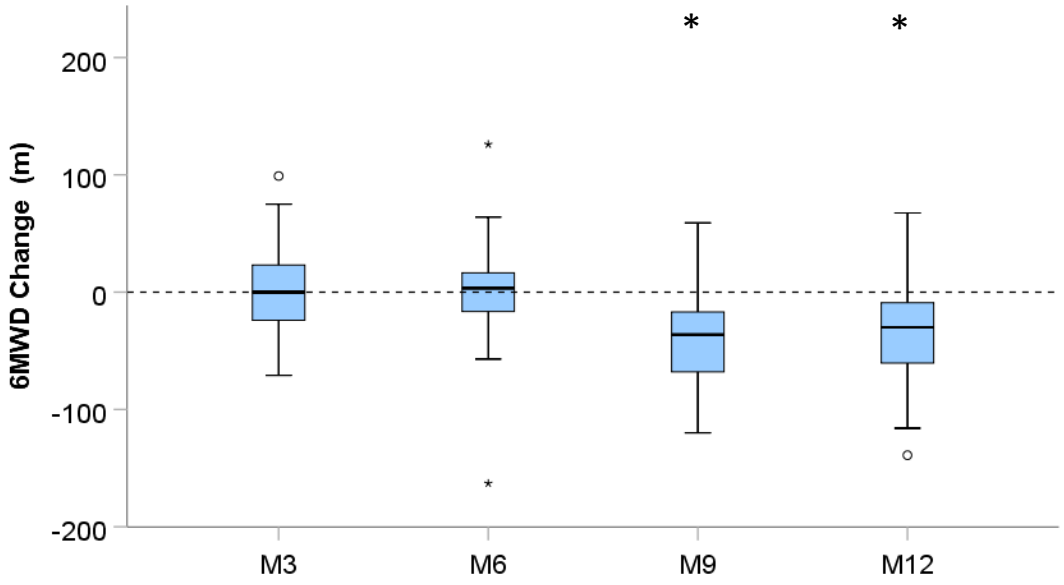
DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 17: 6MWD Change Over Time in Patients with DMD



DMD = Duchenne muscular dystrophy

*Means that the median of the 6MWD change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, a decline in the median change from baseline scores was observed at each time point (most notably after 9 months of follow-up) in the 8 to 14 years age category, while a decrease in 6MWD scores was reported only after 9 months of follow-up in patients 5 to 7 years of age. Statistically significant median score changes were only observed at Months 9 and 12 for the 8 to 14 years of age group (P-values = 0.001 and 0.009, respectively). The lack of statistical significance in the younger population may have to do with the small sample size at each time point (the number of patients ranged from n = 13 at Month 3 to n = 6 at Month 12).

Of note, a larger decrease was observed in patients 8 to 14 years of age (median change from baseline scores ranged from -2.5 m at Month 3 to -41.5 m at Month 12) compared with patients aged 5 to 7 years of age (median change from baseline scores ranged from 6.0 m at Month 3 to -18.0 m at Month 12). The difference in changes in 6MWD between groups did not reach the statistical significance based on the Mann-Whitney U test, with the exception of after 6 months of follow-up where in the

youngest patients their 6MWD seemed to improve (Table 45 and Figure 18).

Table 45: 6MWD Change Over Time in Patients with DMD (Stratified by Age Group)

6MWD Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	13	30	13	22	8	16	6	14
Median (m)	6.0	-2.5	12.0	-1.0	-30.3	-45.5	-18.0	-41.5
Mean (m)	5.8	-4.0	23.1	-11.9	-34.2	-42.3	-28.3	-41.6
SD (m)	32.5	38.6	42.5	41.0	58.4	31.1	59.0	44.7
P-value*	0.507	0.449	0.087	0.346	0.161	**0.001**	0.249	**0.009**
SRM**	-	-	-	-	-	1.36	-	0.93
Sample size estimated α = 5% β = 20%	-	-	-	-	-	8	-	18
Sample size estimated α = 5% β = 10%	-	-	-	-	-	11	-	24
Sample size estimated α = 5% β = 5%	-	-	-	-	-	14	-	30
P-value***	0.288		**0.031**		0.610		0.353	

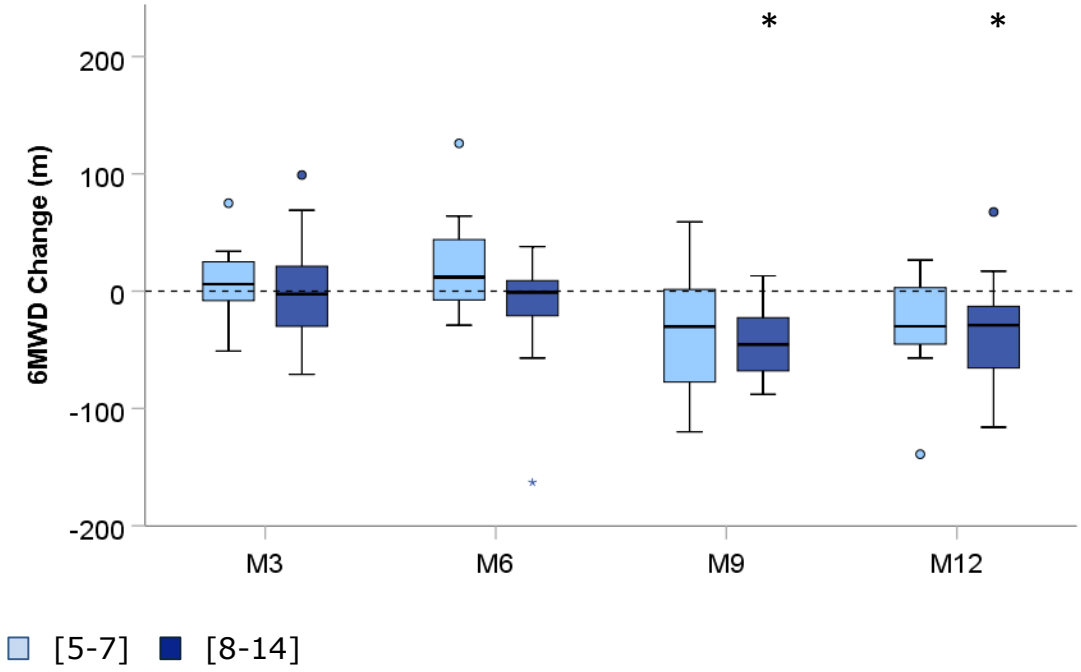
DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD – $n=2*\Phi^2/SRM^2$ with Φ = 2.802; 3.242; 3.605 for α = 5% - β = 20%; α = 5% - β = 10%; and α = 5% - β = 5% respectively.

***Mann-Whitney U test to detect any age-range effect.

Figure 18: 6MWD Change Over Time in Patients with DMD (Stratified by Age Groups)



6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy

*Means that the median of the 6MWD change is statistically different from zero (One-sample Wilcoxon signed rank test)

The SV95C median change scores were calculated using the same population to assess 6MWD. Overall, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.040 m/s -0.080 m/s, -0.110 m/s, and -0.170 m/s, respectively), with statistically significant median score changes observed at each timepoint (P-values ranged from 0.001 to 0.008). These results indicate that the SV95C is more sensitive to detect disease progression over the course of 12 months than the 6MWD. It allows an earlier detection with fewer patients.

When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for the 8 to 14 years of age group, indicating that the loss of the maximal speed was progressive over time. Statistically significant median score changes were also observed at each timepoint (P-values < 0.05). For the 5 to 7 year age group, a decline was observed at each timepoint, most notably after 9 months of follow-up. The lack of statistical significance in the 5 to 7 year age group could possibly be due to the low sample size at each time point. The decrease in SV95C change scores were generally comparable between age groups, with no statistical significance observed at any time point based on the Mann-Whitney U test to detect any age-range effect (P-values > 0.05; Table 46 and Table 47).

Table 46: SV95C Change Over Time in Patients with DMD (Based on the Same Sample Used to Calculate the 6MWD)

SV95C Change	3M	6M	9M	12M
N	43	35	24	20
Median (m/s)	-0.040	-0.080	-0.110	-0.170

SV95C Change	3M	6M	9M	12M
Mean (m/s)	-0.067	-0.087	-0.132	-0.229
SD (m/s)	0.124	0.131	0.218	0.263
P-value*	**0.001**	**0.001**	**0.008**	**0.002**
SRM**	0.54	0.66	0.61	0.87
Sample size estimated α = 5% β = 20%	53	36	43	21
Sample size estimated α = 5% β = 10%	71	48	57	28
Sample size estimated α = 5% β = 5%	88	59	71	34

6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = $2*\Phi^2/SRM^2$ with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Table 47: SV95C Change Over Time in Patients with DMD by Age Group (Based on the Same Sample Used to Calculate the 6MWD)

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	13	30	13	22	8	16	6	14
Median (m/s)	-0.080	-0.030	-0.033	-0.095	-0.110	-0.115	-0.142	-0.170
Mean (m/s)	-0.077	-0.063	-0.089	-0.085	-0.133	-0.132	-0.272	-0.210
SD (m/s)	0.164	0.105	0.151	0.122	0.248	0.211	0.336	0.237
P-value*	0.151	**0.002**	0.116	**0.006**	0.141	**0.026**	0.080	**0.011**
SRM**		0.60		0.70		0.63		0.88
Sample size estimated α = 5% β = 20%		44		32		40		20
Sample size estimated α = 5% β = 10%		58		43		54		27
Sample size estimated α = 5% β = 5%		72		53		66		33
P-value***	0.845		0.906		0.976		0.718	

6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5% - β = 20%; α = 5% - β = 10%; and α = 5% - β = 5% respectively.

*** Mann-Whitney U test to detect any age-range effect.

NSAA

As for the 6MWD, for the NSAA, a decline in the median change from baseline scores was reported only after 9 months of follow-up (median change from baseline scores were 0.00, 1.00, -1.00, and -2.00 at Months 3, 6, 9, and 12,respectively), with statistically significant median score changes observed at Months 9 and 12 (P-values = 0.026 and 0.016, respectively; Table 48 and Figure 19).

Table 48: NSAA Change Over Time in Patients with DMD

NSAA Change	3M	6M	9M	12M
N	46	21	26	15
Median (#)	0.00	1.00	-1.00	-2.00
Mean (#)	0.39	0.71	-1.92	-2.27
SD (#)	2.454	2.028	3.867	3.011
P-value*	0.373	0.114	**0.026**	**0.016**
SRM**	-	-	0.50	0.75
Sample size estimated α = 5% - β = 20%	-	-	64	28
Sample size estimated α = 5% - β = 10%	-	-	85	37
Sample size estimated α = 5% - β = 5%	-	-	105	46

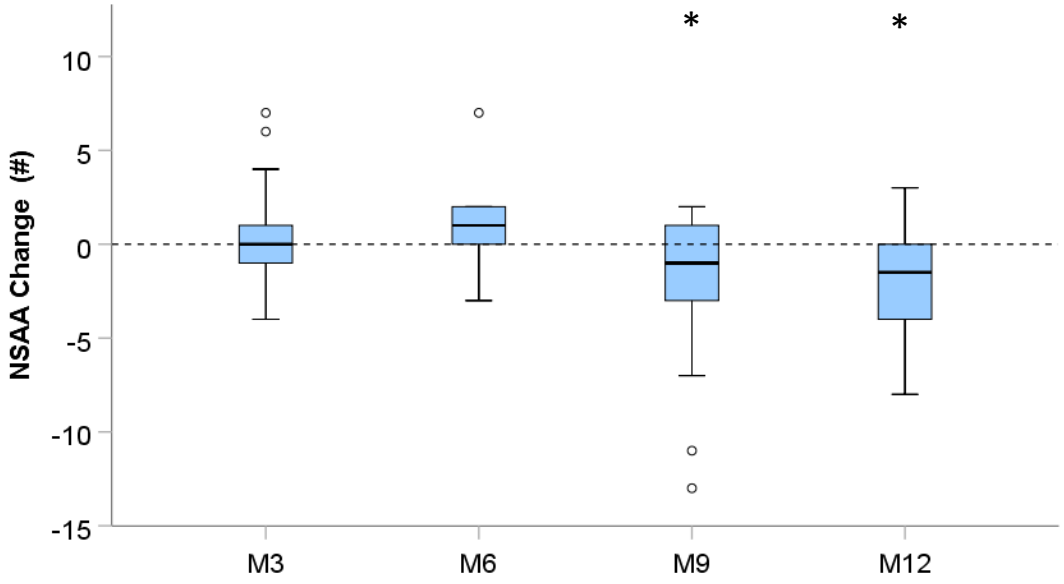
DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5% - β = 20%; α = 5% - β = 10%; and α = 5% - β = 5% respectively.

Figure 19: NSAA Change Over Time in Patients with DMD



DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment

*Means that the median of the NSAA change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, a decrease in NSAA scores was reported only after 9 months of follow-up in the 8 to 14 years of age group, while generally stable scores were reported for the 5-to-7-year age group. Of note, a lower score was observed in patients 8 to 14 years of age (median change from baseline scores ranged from 0.00 at Month 3 to -3.00 at Month 12) compared with patients aged 5 to 7 years (median change from baseline scores ranged from 0.50 at Month 3 to 0.00 at Month 12). Statistically significant decrease in the median score changes were observed at Months 9 and 12 for the 8 to 14 years of age group (P-values = 0.018 and 0.011, respectively) and a statistically significant increase at Month 6 for the 5 to 7 years of age group (P-value = 0.040). The change in NSAA did not reach statistical significance based on the Mann-Whitney U test to detect any age-range effect (P > 0.05; Table 49 and Figure 20).

Table 49: NSAA Change Over Time in Patients with DMD (Stratified by Age Group)

NSAA Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	14	32	7	14	9	17	5	10
Median (#)	0.50	0.00	2.00	0.00	0.00	-1.00	0.00	-3.00
Mean (#)	1.29	0.00	2.00	0.07	-0.56	-2.65	-1.00	-2.90
SD (#)	2.785	2.229	2.449	1.492	2.651	4.271	4.183	2.234
P-value*	0.121	0.989	**0.040**	0.809	0.670	**0.018**	0.854	**0.011**
SRM**	-	-	0.82	-	-	0.62	-	1.30
Sample size estimated α = 5% β = 20%	-	-	24	-	-	41	-	9
Sample size estimated α = 5% β = 10%	-	-	32	-	-	55	-	12
Sample size estimated α = 5% β = 5%	-	-	39	-	-	68	-	15
P-value***	0.179		0.056		0.220		0.206	

DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SRM = standardized response mean

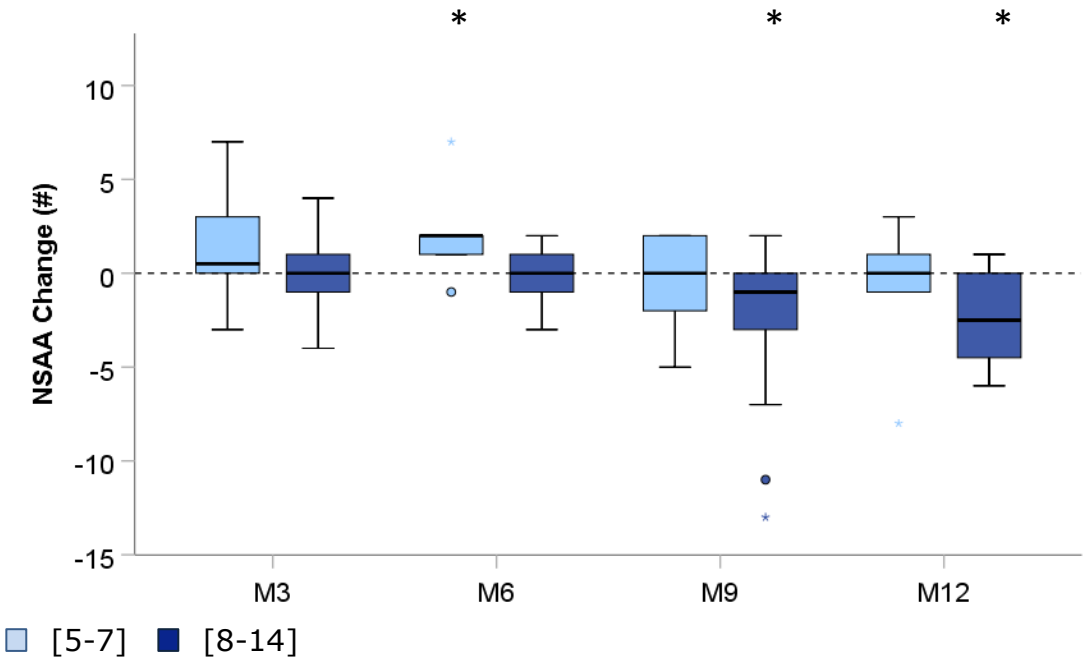
*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ^2/SRM^2 with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

*** Mann-Whitney U test to detect any age-range effect.

Figure 20: NSAA Change Over Time in Patients with DMD (Stratified by Age Groups)



DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment

*Means that the median of the NSAA change is statistically different from zero (One-sample Wilcoxon signed rank test)

The SV95C median change scores were calculated using the same population to assess the NSAA. Overall, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.035, -0.060, -0.110, and -0.130, respectively), with statistically significant median score changes observed at each timepoint (P-values ranged from 0.003 to 0.036). These results indicate that the SV95C is more sensitive to detect disease progression over the course of 12 months than the NSAA. It allows an earlier detection with fewer patients.

When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for the 8 to 14 years of age group, indicating that the loss of the maximal speed was progressive over time. Statistically significant median score changes were observed at each timepoint (P-values < 0.05). For the 5 to 7 year age group, a decline was observed at each timepoint (except for after 6 months of follow-up). The lack of statistical significance in the 5 to 7 year age group could possibly be due to the low sample size at each time point. Of note, a larger decrease was observed at most time points in patients 8 to 14 years of age (median change from baseline scores ranged from -0.030 at Month 3 to -0.170 at Month 12) than in patients aged 5 to 7 years of age (median change from baseline scores ranged from -0.065 at Month 3 to -0.050 at Month 12). However, no statistically significant difference observed at any time point based on the Mann-Whitney U test to detect any age-range effect (P-values > 0.05; Table 50 and Table 51).

Table 50: SV95C Change Over Time in Patients with DMD (Based on the Same Sample Used to Calculate the NSAA)

SV95C Change	3M	6M	9M	12M
N	46	21	26	15
Median (m/s)	-0.035	-0.060	-0.110	-0.130
Mean (m/s)	-0.062	-0.072	-0.125	-0.250
SD (m/s)	0.123	0.135	0.216	0.288
P-value*	**0.003**	**0.036**	**0.009**	**0.006**
SRM**	0.50	0.54	0.58	0.87
Sample size estimated α = 5% β = 20%	62	55	47	21
Sample size estimated α = 5% β = 10%	84	73	63	28
Sample size estimated α = 5% β = 5%	103	90	77	35

DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Table 51: SV95C Change Over Time in Patients with DMD by Age Group (Based on the Same Sample Used to Calculate the NSAA)

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	14	32	7	14	9	17	5	10
Median (m/s)	-0.065	-0.030	0.020	-0.095	-0.110	-0.120	-0.050	-0.170
Mean (m/s)	-0.067	-0.059	-0.050	-0.084	-0.104	-0.136	-0.280	-0.235
SD (m/s)	0.162	0.104	0.170	0.119	0.247	0.205	0.375	0.256
P-value*	0.198	**0.003**	0.866	**0.025**	0.285	**0.015**	0.144	**0.022**
SRM**	-	0.57	-	0.70	-	0.66	-	0.92
Sample size estimated	-	49	-	32	-	36	-	19

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
α = 5% β = 20%								
Sample size estimated α = 5% β = 10%	-	65	-	43	-	48	-	25
Sample size estimated α = 5% β = 5%	-	81	-	53	-	59	-	31
P-value***	0.981		0.400		0.220		1.000	

DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

*** Mann-Whitney U test to detect any age-range effect.

4SC

For the 4SC, the time required to climb 4 stairs increased at each time point, most notably after 9 and 12 months of follow-up (median change from baseline scores were 0.750, 0.200, 0.520, and 1.500 at Months 3, 6, 9, and 12, respectively), with statistically significant median score changes observed at each time point (P-values < 0.05; Table 52 and Figure 21).

In addition, based on the estimated sample size, the number of subjects required to identify an increase in 4SC remains high, even after 12 months of follow-up (Table 52).

Table 52: 4SC Change Over Time in Patients with DMD

4SC Change	3M	6M	9M	12M
N	46	21	26	15
Median (s)	0.08	0.20	0.52	1.50
Mean (s)	0.65	0.61	0.96	2.12
SD (s)	2.16	1.22	1.78	3.50
P-value*	**0.027**	**0.023**	**0.009**	**0.031**
SRM**	0.30	0.50	0.54	0.61
Sample size estimated α = 5% β = 20%	175	64	55	43
Sample size estimated	235	86	73	57

α = 5% β = 10%				
Sample size estimated α = 5% β = 5%	290	106	90	71

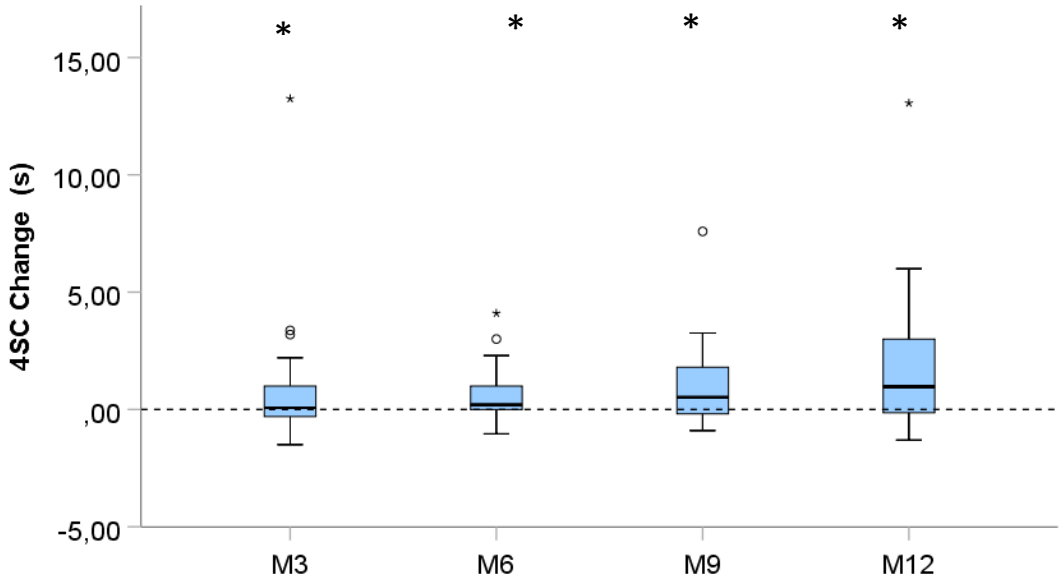
4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 21: 4SC Change Over Time in Patients with DMD



4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy

*Means that the median of the 4SC change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, the time needed to climb 4 stairs did not increase continuously over time. The largest increase in median change 4SC scores was observed at 9 and 12 months of follow-up for the 8 to 14 years of age group, with statistically significant median score changes only observed at Months 3 and 9 (P-values = 0.033 and 0.007, respectively; however, the statistical significance at 3 months follow-up was most likely due to outliers). In addition, the change in 4SC did not reach the statistical based on the Mann-Whitney U test to detect any age-range effect (P > 0.05; Table 53 and Figure 22).

Table 53: 4SC Change Over Time in Patients with DMD (Stratified by Age Group)

4SC Change	3M		6M		9M			12M
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	14	32	7	14	9	17	5	10
Median (s)	-0.01	0.31	0.21	0.15	0.00	1.00	0.28	1.75
Mean (s)	0.22	0.83	0.97	0.43	0.16	1.38	1.29	2.54
SD (s)	0.81	2.52	1.71	0.92	0.72	2.04	1.86	4.12
P-value*	0.463	**0.033**	0.150	0.075	0.779	**0.007**	0.138	0.074
SRM**		0.33				0.67		
Sample size estimated α=5% β=20%		144				35		
Sample size estimated α=5% β=10%		192				46		
Sample size estimated α=5% β=5%		238				57		
P-value***	0.466		0.535		0.095			1.000

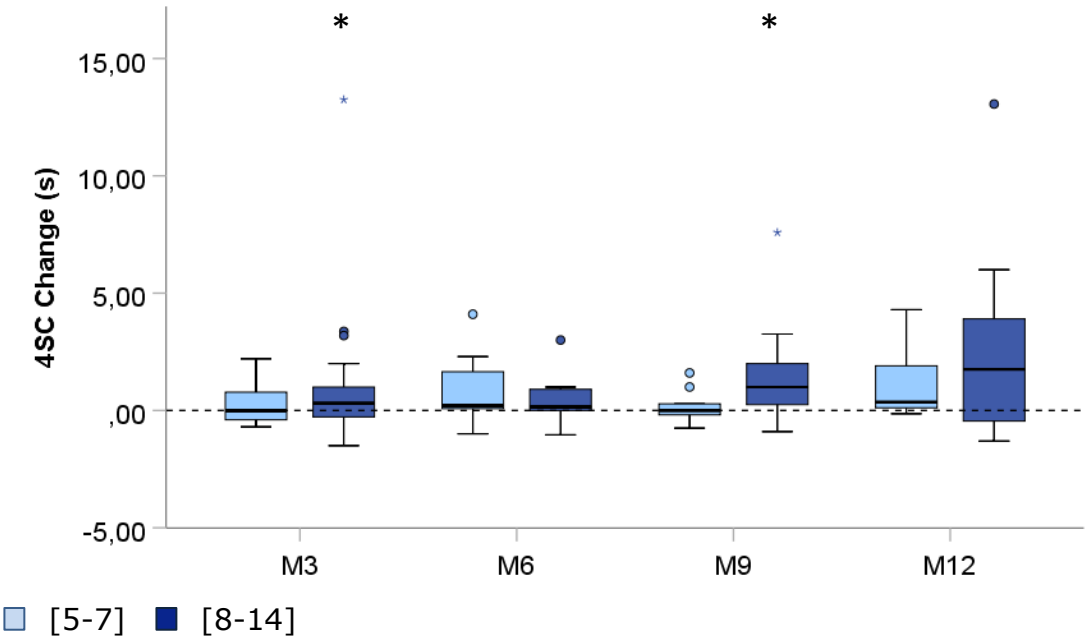
4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = $2*\Phi^2/SRM^2$ with Φ = 2.802; 3.242; 3.605 for α = 5% - β = 20%; α = 5% - β = 10%; and α = 5% - β = 5% respectively.

Figure 22: 4SC Change Over Time in Patients with DMD (Stratified by Age Groups)



4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy

*Means that the median of the 4SC change is statistically different from zero (One-sample Wilcoxon signed rank test)

The SV95C median change scores were calculated using the same population to assess the 4SC. Overall, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.035, -0.060, -0.110, and -0.130, respectively), with statistically significant median score changes observed at each timepoint (P-values ranged from 0.003 to 0.036). These results indicate that the SV95C is more sensitive to detect disease progression over the course of 12 months than the 4SC. It allows an earlier detection with fewer patients.

When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for the 8 to 14 years of age group, indicating that the loss of the maximal speed was progressive over time. Statistically significant median score changes observed at each timepoint (P-values < 0.05). For the 5 to 7 years of age group, a decline was observed at most time points, except for after 6 months of follow-up. The lack of statistical significance in the 5 to 7 year age group could possibly be due to the low sample size at each time point. In addition, no statistical significance was observed at any time point based on the Mann-Whitney U test to detect any age-range effect (P-values > 0.05; Table 54 and Table 55).

Table 54: SV95C Change Over Time in Patients with DMD (Based on the Same Sample Used for the 4SC)

SV95C Change	3M	6M	9M	12M
N	46	21	26	15
Median (m/s)	-0.035	-0.060	-0.110	-0.130
Mean (m/s)	-0.062	-0.072	-0.125	-0.250
SD (m/s)	0.123	0.135	0.216	0.288

SV95C Change	3M	6M	9M	12M
P-value*	**0.003**	**0.036**	**0.009**	**0.006**
SRM**	0.50	0.54	0.58	0.87
Sample size estimated α = 5% β = 20%	62	55	47	21
Sample size estimated α = 5% β = 10%	84	73	63	28
Sample size estimated α = 5% β = 5%	103	90	77	35

4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Table 55: SV95C Change Over Time in Patients with DMD by Age Group (Based on the Same Sample Used for the 4SC)

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	14	32	7	14	9	17	5	10
Median (m/s)	-0.065	-0.030	0.020	-0.095	-0.110	-0.120	-0.050	-0.170
Mean (m/s)	-0.067	-0.059	-0.050	-0.084	-0.104	-0.139	-0.280	-0.235
SD (m/s)	0.162	0.104	0.170	0.119	0.247	0.205	0.375	0.256
P-value*	0.198	**0.003**	0.866	**0.025**	0.285	**0.015**	0.144	**0.022**
SRM**	-	0.57	-	0.70	-	0.66	-	0.92
Sample size estimated α = 5% β = 20%	-	49	-	32	-	36	-	19
Sample size estimated α = 5% β = 10%	-	65	-	43	-	48	-	25

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
Sample size estimated α = 5% β = 5%	-	81	-	53	-	59	-	31
P-value***	0.981		0.400		0.220		1.000	

4SC = 4-stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

*** Mann-Whitney U test to detect any age-range effect.

3.2.2.4.3. Natural Change Over Time (17 Patients Followed Over 12 Months)

SV95C

Responsiveness of the SV95C was also determined from a set of 17 patients who were followed over 12 months. Despite the small sample size, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported (median change from baseline scores were -0.043, -0.067, -0.157, and -0.197, respectively), with statistically significant median score changes observed at Months 6, 9, and 12 (P-values < 0.01; Table 56 and Figure 23).

Table 56: SV95C Change Over Time in Patients with DMD (N = 17)

SV95C Change	3M	6M	9M	12M
N	17	17	17	17
Median (m/s)	-0.043	-0.067	-0.157	-0.197
Mean (m/s)	-0.049	-0.078	-0.179	-0.225
SD (m/s)	0.121	0.126	0.211	0.241
P-value*	0.210	**0.031**	**0.003**	**0.004**
SRM**	-	0.62	0.85	0.94
Sample size estimated α = 5% β = 20%	-	41	22	18
Sample size estimated α = 5% β = 10%	-	55	29	24
Sample size estimated α = 5% β = 5%	-	68	36	30

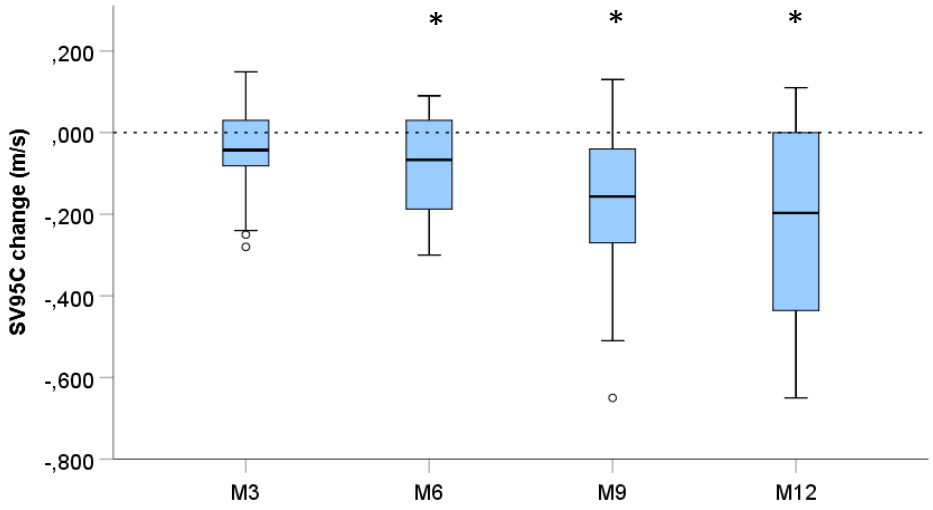
DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 23: SV95C Change Over Time in Patients with DMD (N = 17)



DMD = Duchenne muscular dystrophy; SV95C = 95th centile of the stride velocity

*Means that the median of the SV95C change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, a continual decline in the median change from baseline scores at Month 3 to Month 12 was reported for each group, although statistical significance was only reached at 9- and 12-months follow-up in the younger age population. Of note, a larger decrease was observed in patients 8 to 14 years of age (median change from baseline scores ranged from -0.046 at Month 3 to -0.389 at Month 12) compared with patients aged 5 to 7 years (median change from baseline scores ranged from 0.010 at Month 3 to -0.137 at Month 12; Table 57 and Figure 24).

Table 57: SV95C Change Over Time in 17 Patients with DMD (Stratified by Age Group)

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	9	8	9	8	9	8	9	8
Median (m/s)	0.010	-0.046	-0.067	-0.127	-0.147	-0.226	-0.137	-0.389
Mean (m/s)	-0.017	-0.085	-0.052	-0.106	-0.147	-0.215	-0.168	-0.289
SD (m/s)	0.116	0.124	0.099	0.152	0.147	0.272	0.173	0.299
P-value*	0.953	0.093	0.139	0.161	**0.011**	0.069	**0.017**	0.093

SV95C Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
SRM**	-	-	-	-	1.00	-	0.97	-
Sample size estimated α = 5% β = 20%	-	-	-	-	16	-	17	-
Sample size estimated α = 5% β = 10%	-	-	-	-	21	-	22	-
Sample size estimated α = 5% β = 5%	-	-	-	-	26	-	27	-

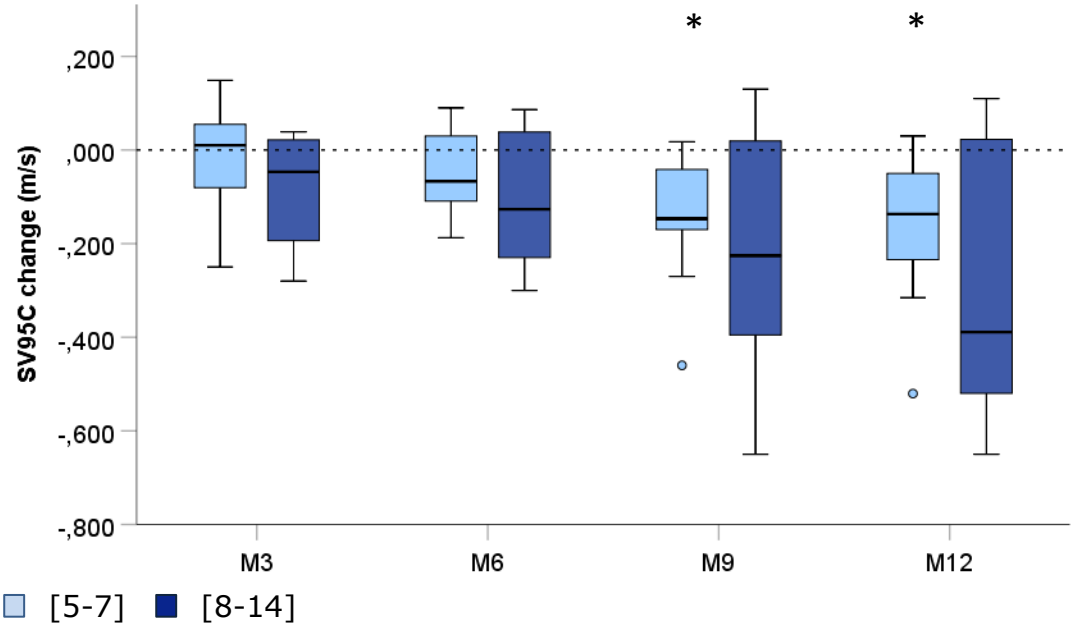
DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 24: SV95C Change Over Time in 17 Patients with DMD (Stratified by Age Groups)



DMD = Duchenne muscular dystrophy; SV95C = 95th centile of the stride velocity

6MWD

For the 6MWD, a decline in the median change from baseline scores was reported after 6 months of

follow-up (median change from baseline scores were 25.0, -9.5, -56.0, and -30.0 at Months 3, 6, 9, and 12, respectively); no statistically significant differences were observed at any time point likely due to the small sample size at each time point (Table 58 and Figure 25).

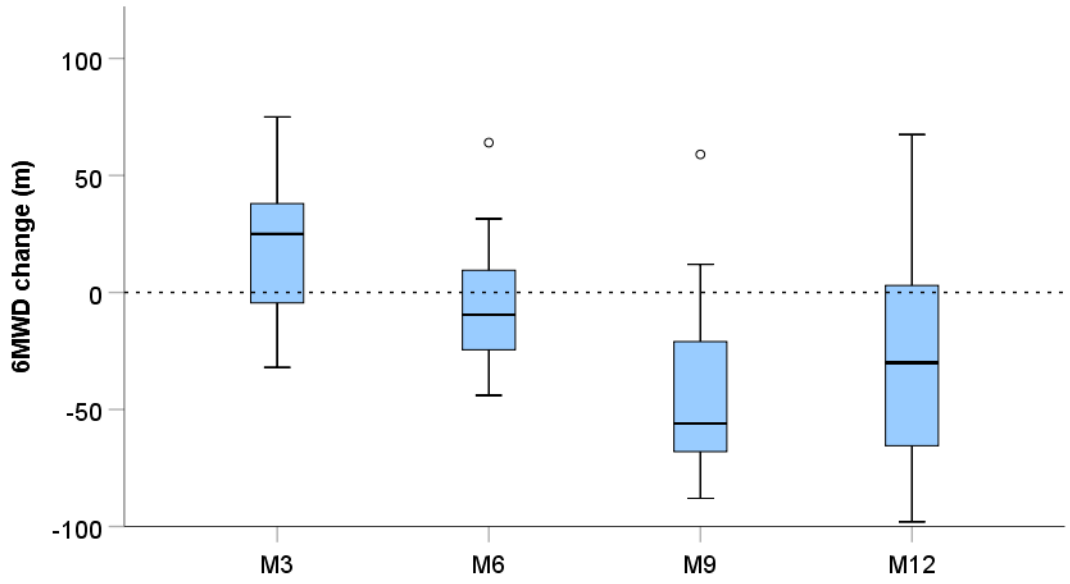
Table 58: 6MWD Change Over Time in Patients with DMD (N = 7 at Months 3 and 9 and N = 11 at Months 6 and 12)

6MWD Change	3M	6M	9M	12M
N	7	11	7	11
Median (m)	25	-9.5	-56	-30
Mean (m)	19.29	-4.182	-37.57	-26.909
SD (m)	36.11	31.803	52.924	49.831
P-value*	0.31	0.477	0.128	0.12

6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy; SD = standard deviation

*One-sample Wilcoxon signed rank test

Figure 25: 6MWD Change Over Time in Patients with DMD (N = 7 at Months 3 and 9 and N = 11 at Months 6 and 12)



6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy

*Means that the median of the 6MWD change is statistically different from zero (One-sample Wilcoxon signed rank test).

When stratified by age group, a decline in the median change from baseline scores was observed at each time point (most notably after months of follow-up) in the 8 to 14 years age category, while the youngest patients appear to have a stable 6MWD over 12 months. No statistically significant differences were observed at any time point for either age group (P-values > 0.05), most likely due to the small sample size (Table 59 and Figure 26).

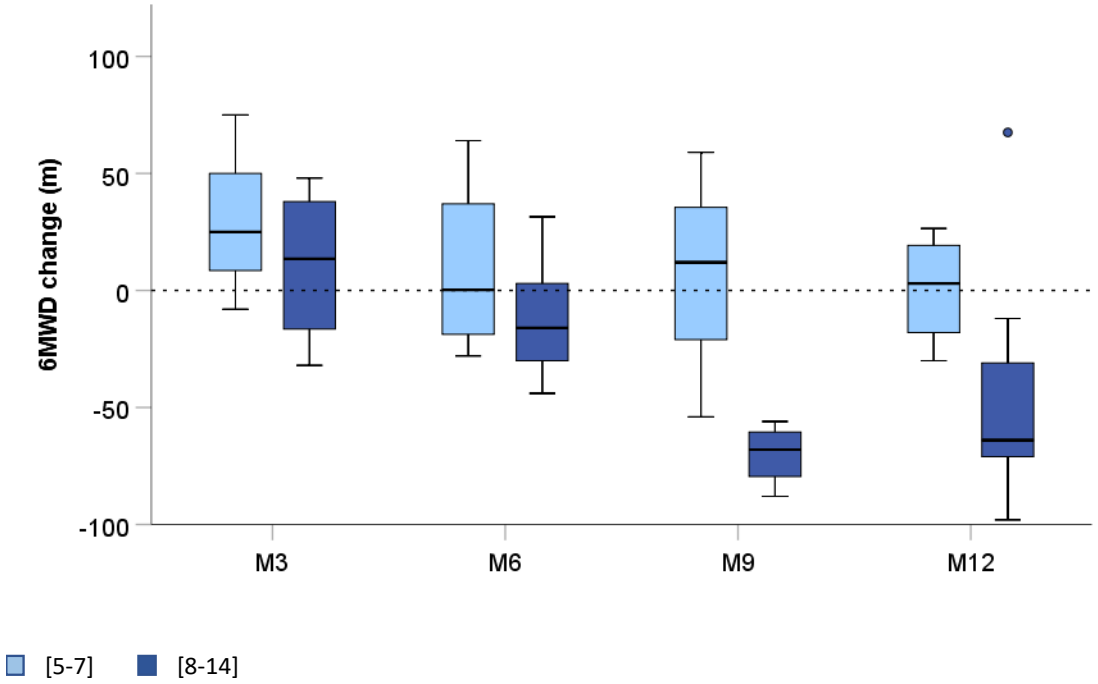
Table 59: 6MWD Change Over Time in Patients with DMD (Stratified by Age Group)

6MWD Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	3	4	4	7	3	4	4	7
Median (m)	25	13.5	0.25	-16	12	-68	3	-64
Mean (m)	30.7	10.8	9.1	-11.8	5.7	-70.0	0.6	-42.6
SD (m)	41.8	34.9	39.7	26.7	56.8	13.5	24.4	55.2
P-value*	0.285	0.715	0.715	0.237	0.593	0.068	1	0.128

6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy; SD = standard deviation

*One-sample Wilcoxon signed rank test

Figure 26: 6MWD Change Over Time in Patients with DMD (Stratified by Age Groups)



6MWD = 6-minute walking distance; DMD = Duchenne muscular dystrophy

*Means that the median of the 6MWD change is statistically different from zero (One-sample Wilcoxon signed rank test)

NSAA

For the NSAA, a small decline in the median change from baseline scores was reported over 12 months of follow-up (median change from baseline scores were 2.0, 1.0, 1.0, and 0.0 at Months 3, 6, 9, and 12, respectively), with statistically significant increase of median score changes observed at Months 3 and 6 (P-values = 0.026 and 0.038, respectively) which was not expected in DMD population. (See Table 60 and Figure 27).

Table 60: NSAA Change Over Time in Patients with DMD (N = 7)

NSAA Change	3M	6M	9M	12M
N	7	7	7	7
Median (#)	2	1	1	0
Mean (#)	1.86	1.14	0.57	-0.29
SD (#)	1.215	0.9	1.272	2.563
P-value*	0.026	0.038	0.234	0.892
SRM**	1.53	1.27	-	-
Sample size estimated α = 5% β = 20%	7	10		
Sample size estimated α = 5% β = 10%	9	13		
Sample size estimated α = 5% β = 5%	11	16		

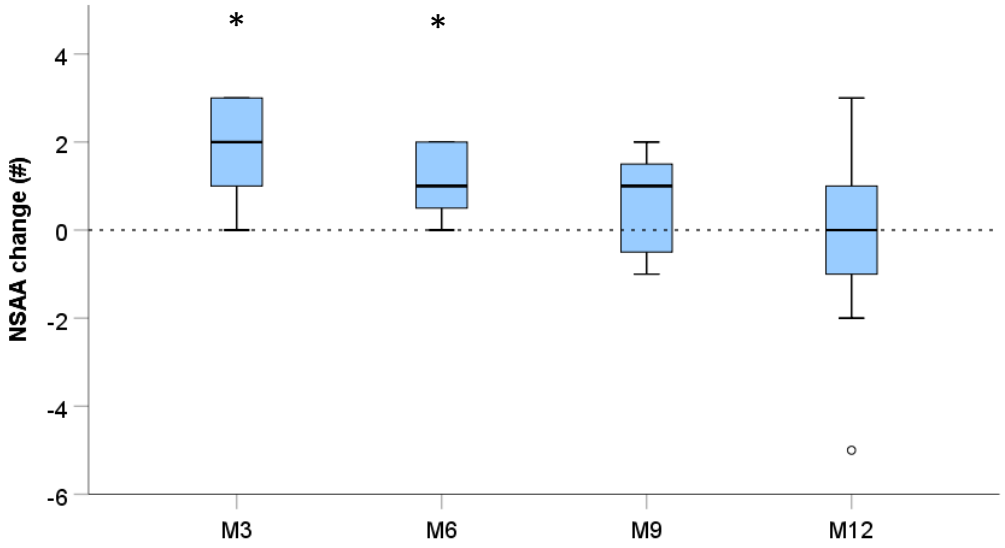
DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SRM = standardized response mean

*One-sample Wilcoxon signed rank test

**SRM = |Mean| / SD

n = 2*Φ²/SRM² with Φ = 2.802; 3.242; 3.605 for α = 5%, β = 20%; α = 5%, β = 10%; and α = 5%, β = 5% respectively.

Figure 27: NSAA Change Over Time in Patients with DMD (N = 7)



DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment

*Means that the median of the NSAA change is statistically different from zero (One-sample Wilcoxon signed rank test)

When stratified by age group, a continual decline in the median change from baseline scores was observed in the 8 to 14 years age category (median scores ranged from 2.5 at Month 3 to -1.0 at Month 12), while the youngest patients appeared to have a stable NSAA over the natural course of 12 months. No statistically significant differences were observed at any time point for either age group (P-values > 0.05), most likely due to the small sample size (Table 61 and Figure 28).

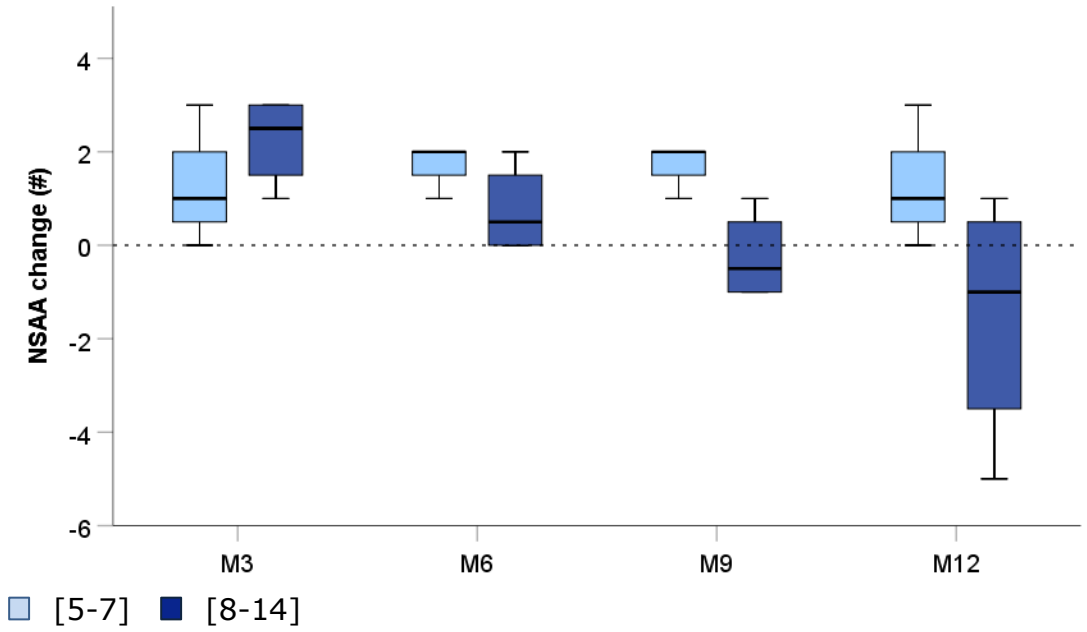
Table 61: NSAA Change Over Time in Patients with DMD (Stratified by Age Group))

NSAA Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	3	4	3	4	3	4	3	4
Median (#)	1	2.5	2	0.5	2	-0.5	1	-1
Mean (#)	1.33	2.25	1.67	0.75	1.67	-0.25	1.33	-1.5
SD (#)	1.528	0.957	0.577	0.957	0.577	0.957	1.528	2.646
P-value*	0.18	0.066	0.102	0.18	0.102	0.564	0.18	0.285

DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; SD = standard deviation

*One-sample Wilcoxon signed rank test

Figure 28: NSAA Change Over Time in Patients with DMD (Stratified by Age Groups)



DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment

*Means that the median of the NSAA change is statistically different from zero (One-sample Wilcoxon signed rank test)

4SC

For the 4SC, the time required to climb 4 stairs started to increase slightly after 9 months of follow-up (median change from baseline scores were 0.5, 0.0, 0.06, and 1.5 at Months 3, 6, 9, and 12, respectively); no statistically significant changes observed at any time point (P-values > 0.05; Table 62 and Figure 29).

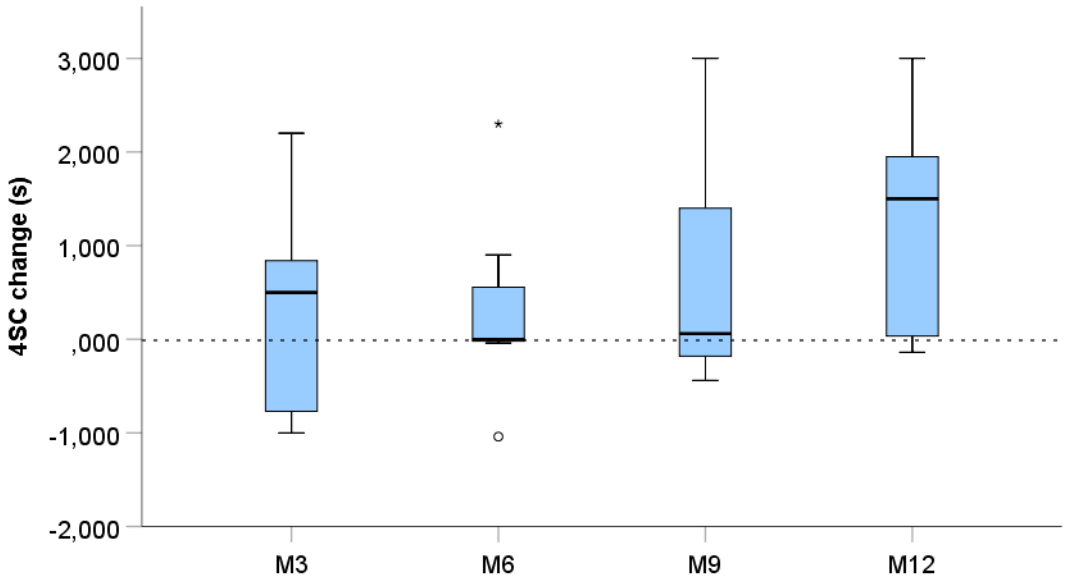
Table 62: 4SC Change Over Time in Patients with DMD (N = 7)

4SC Change	3M	6M	9M	12M
N	7	7	7	7
Median (s)	0.5	0	0.06	1.5
Mean (s)	0.26	0.33	0.72	1.19
SD (s)	1.17	1.04	1.28	1.22
P-value*	0.735	0.5	0.398	0.091

4SC = 4 stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation

*One-sample Wilcoxon signed rank test

Figure 29: 4SC Change Over Time in Patients with DMD (N = 7)



4SC = 4 stair climb test; DMD = Duchenne muscular dystrophy

When stratified by age group, the time needed to climb 4 stairs continuously increased over time for patients aged 8 to 14 years, while the time needed to climb 4 stairs remained stable over the course of 12 months in the younger population (5 to 7 years of age). No statistically significant differences were observed at any time point for either age group most likely due to the small sample size at each time point (P-values > 0.05; Table 63 and Figure 30).

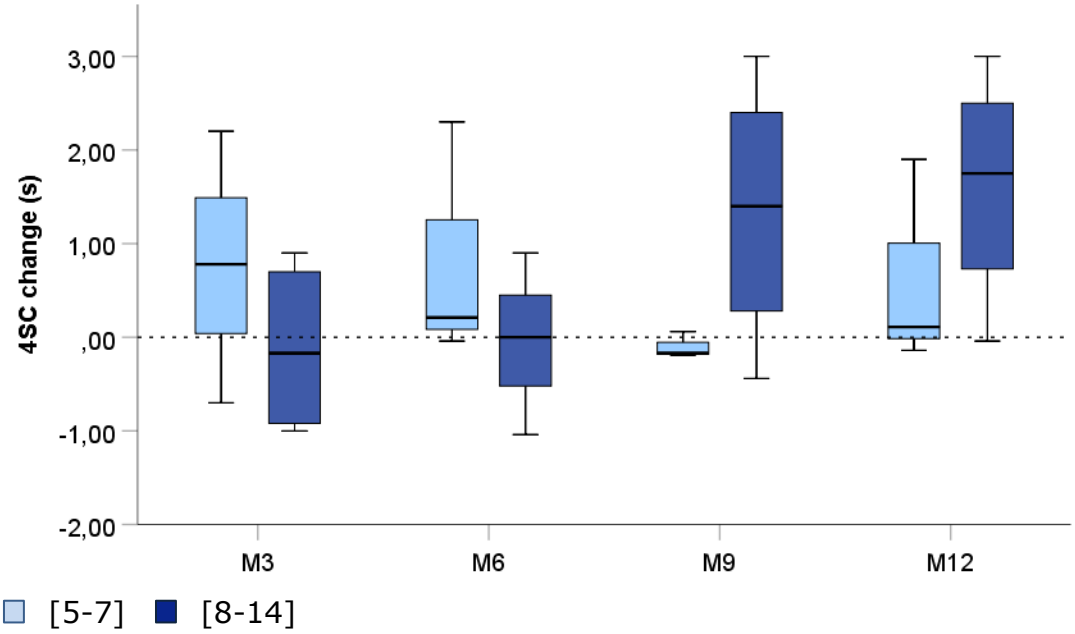
Table 63: 4SC Change Over Time in Patients with DMD (Stratified by Age Group)

4SC Change	3M		6M		9M		12M	
	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]	[5 – 7]	[8 - 14]
N	3	4	3	4	3	4	3	4
Median (s)	0.78	-0.17	0.21	0.00	-0.17	1.40	0.11	1.75
Mean (s)	0.76	-0.11	0.82	-0.04	-0.10	1.34	0.62	1.62
SD (s)	1.45	0.95	1.28	0.79	0.14	1.44	1.11	1.27
P-value*	0.285	0.715	0.285	0.655	0.285	0.144	0.593	0.144

4SC = 4 stair climb test; DMD = Duchenne muscular dystrophy; SD = standard deviation

*One-sample Wilcoxon signed rank test

Figure 30: 4SC Change Over Time in Patients with DMD (Stratified by Age Groups)



4SC = 4 stair climb test; DMD = Duchenne muscular dystrophy

3.2.2.4.4. Positive changes – Treatment Benefit

The sensitivity of SV95C to a positive change was assessed in 11 patients with DMD who were started on corticosteroids. In this small sample of 11 patients, a significant positive change in SV95C as early as 3 months was observed (which would indicate an improvement in response to treatment; P-value = 0.003). This was confirmed at 6 months based on the median SV95C change scores from baseline to Month 3 and Month 6 (0.090 m/s and 0.211 m/s, respectively). Starting at 9 months, no additional significant positive changes observed although there was further improvement at 12 months which was non-significant most likely because of small sample size. The SRM was above 1 also indicating a high sensitivity to detect positive changes (Table 64 and Figure 31).

Table 64: SV95C Change Over Time in Patients with DMD who Initiated Corticosteroids

SV95C Change	3M	6M	9M	12M
N	11	7	7	5
Median (m/s)	0.090	0.211	0.218	0.307
Mean (m/s)	0.135	0.247	0.208	0.266
SD (m/s)	0.128	0.172	0.233	0.275
P-value*	**0.003**	**0.018**	0.063	0.080
SRM**	1.05	1.44		
Sample size estimated α = 5% β = 20%	14	8		

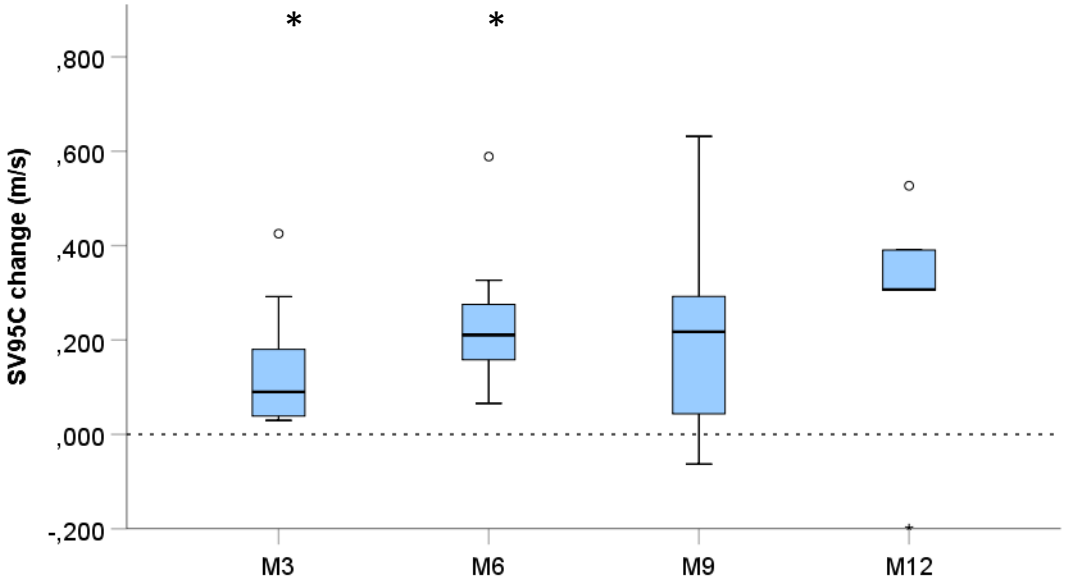
Sample size estimated α = 5% β = 10%	19	10		
Sample size estimated α = 5% β = 5%	23	13		

DMD = Duchenne muscular dystrophy; SD = standard deviation; SRM = standardized response mean; SV95C = 95th centile of the stride velocity

*One-sample Wilcoxon signed rank test

**SRM: SRM = |Mean| / SD – $n=2*\Phi^2/SRM^2$ with Φ= 2,802 ; 3,242 ; 3,605 for α=5%, β=20% ; α=5%, β=10% ; and α=5%, β=5% respectively.

Figure 31: SV95C Change Over Time in Patients with DMD who Initiated Corticosteroids



DMD = Duchenne muscular dystrophy; SV95C = 95th centile of the stride velocity

*Means that the median of the SV95C change is statistically different from zero (One-sample Wilcoxon signed rank test)

Overall, SV95C appears able to detect change in the ambulatory capabilities of DMD patients as early as 3 months and would require smaller sample size to detect a significant change, making it more sensitive to change than existing COAs.

3.2.2.5. Meaningful Change Thresholds

3.2.2.5.1. Distribution-based Threshold

The measurement error, SEM (refer to Section 3.1.2.5.1) was calculated as 0.070 m/s for the DMD population in patients aged 5 to 14 years and 0.156 m/s for the control population in subjects aged 6 to 14 years, showing a higher variability in measurement in the control subject probably linked to a higher variability in their stride velocity likely because they are not limited by the disease and may

undertake a variety of activities. The MDC of SV95C at the 80% confidence level was 0.127 m/s and 0.282 m/s, respectively. Similar results were observed when the age group was stratified by younger (5 to 7 years in the DMD population and 6 to 7 years in the control population) and older populations (8 to 14 years of age for both groups; Table 65). The half-SD estimate for SV95C in the DMD population was calculated as 0.191 m/s (Table 66). These values suggest that in DMD patients a change of between ≈0.1 and ≈0.2 m/s would be beyond measurement error.

Table 65: SV95C MCID and MDC in DMD Patients

	DMD			CTRL		
	[5 - 14]	[5 - 7]	[8 - 14]	[6 - 14]	[6 - 7]	[8 - 14]
N	103	43	60	55	17	38
ICC*	0.962	0.956	0.957	0.851	0.814	0.862
95% CI	[0.943 – 0.974]	[0.918 – 0.976]	[0.928 – 0.974]	[0.744 – 0.913]	[0.500 – 0.932]	[0.735 – 0.928]
SV95C-RP1 mean (m/s)	1.467	1.609	1.365	2.383	2.266	2.435
SV95C-RP1 SD	0.360	0.338	0.342	0.404	0.321	0.429
SEM (m/s)**	**0.070**	**0.071**	**0.071**	**0.156**	**0.139**	**0.159**
SEM relative to RP1 (%)	4.780	4.412	5.193	6.539	6.113	6.545
MDC80% (m/s)	**0.127**	**0.129**	**0.129**	**0.282**	**0.251**	**0.289**
MDC90% (m/s)	0.163	0.165	0.165	0.362	0.322	0.371
MDC95% (m/s)	0.194	0.197	0.197	0.432	0.384	0.442

DMD = Duchenne muscular dystrophy; CI = confidence interval; ICC = intra-class correlation; MCID = minimal clinically important difference; MDC = minimal detectable change; RP = recording period; SD = standard deviation; SEM = standard error of measurement; SV95C = 95th centile of the stride velocity

*ICC– 2-way random effect model, absolute agreement, average measure.
**SEM = SD*SQR(1-ICC)

***MDC = z-score*SEM*SQR(2) with z-score = 1.960, 1.645, and 1.282 at 95%, 90%, 80% confidence levels, respectively.

Table 66: Other SV95C Distribution-based Thresholds

Baseline	N	Mean	Median	SD	0.2 SD	0.5 SD	0.8 SD
SV95C (m/s)	125	1.571	1.563	0.382	0.076	0.191	0.305

SD = standard deviation. SV95C = 95th centile of the stride velocity

The MCTs for the 6MWD, NSAA, and 4SC were estimated from MDC thresholds based on a > 80% confidence published on the cTAP-MDA-2021 poster (https://ctap-duchenne.org/wp-content/uploads/2021/03/cTAP-MDA-2021-poster-MDC-analyses-for-functional-outcomes-FINAL.pdf). They were reported to be 20.0 meters, 1.53 units, and 0.72 seconds, respectively. The corresponding

thresholds based on half-SD estimates were calculated as 37.8 meters for 6MWD, 3.1 units for NSAA, and 0.82 seconds for 4SC (Table 67).

SEM relative to baseline of SV95C (4.78 %) was similar to those of 6MWD (5.14%) and lower to those of NSAA (6.73 %) or 4SC (18.72%) indicating a lower variability due to measurement errors with SV95C.

Table 67: 6MWD, NSAA, and 4SC Thresholds

Baseline	N	Mean	Median	SD	*MDC 80%* *	SEM	SEM relative to BL (%)	0.2 SD	0.5 SD	0.8 SD
6MWD (m)	107	390.0	389	75.73	*36.3*	20.0	5.14	15.1	37.9	60.6
NSAA (#)	107	22.9	23	6.33	*2.78*	1.53	6.73	1.3	3.2	5.1
4SC (s)	107	3.82	3.4	1.578	*1.310*	0.72	18.72	0.32	0.79	1.26

4SC = 4 stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; DMD = Duchenne muscular dystrophy; MDC = minimal detectable change; NSAA = North Star Ambulatory Assessment; SD = standard deviation

*: MDC of 6MWD, NSAA, and 4SC were published on the cTAP-MDA-2021 poster (https://ctap-duchenne.org/wp-content/uploads/2021/03/cTAP-MDA-2021-poster-MDC-analyses-for-functional-outcomes-FINAL.pdf). SEM was calculated based on published MDC80%

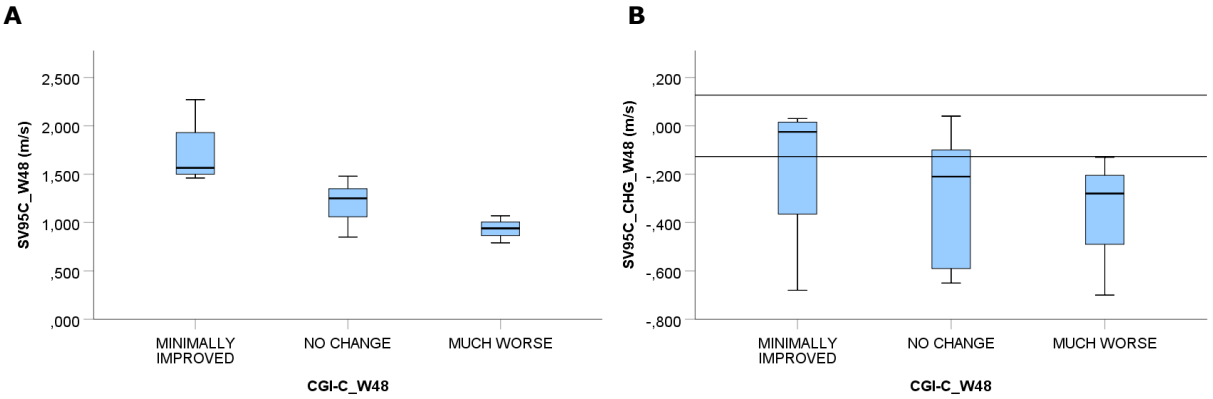
3.2.2.5.2. Anchor-based Within Patient Threshold

Anchor based on patient reported outcome

The SV95C MCT was also assessed through an anchor-based approach using the PODCI and CGI-C scales completed respectively by parents or clinicians of patients with DMD enrolled in the CT-B study which was prematurely stopped due to the absence of efficacy of the investigational medical product. The CGI-C was assessed at Week 48 or during the end of study visit. Only CGI-C assessed at Week 48 was analyzed (N = 12), while PODCI was assessed at Week 48 (N = 15).

Overall, a strong correlation was observed between the SV95C and the CGI-C measured at Week 48 (Spearman correlation coefficient ρ = -0.816, P-value = 0.001; Figure 32-A). There was a clear trend for SV95C scores at Week 48 (SV95C_W48) to be lower the greater the worsening reported on the CGI-C assessed at the same time (CGI-C_W48). While no correlation was found between the change in SV95C and the CGI-C at Week 48, most of patients whom clinicians reported no change or a worsening of the global clinical state of their patients experienced a negative change of SV95C higher than the MDC80% (-0.127 m/s) (Figure 32-B).

Figure 32: Relationship Between the Change in SV95C (in m/s) and the CGI-C After 48 Weeks

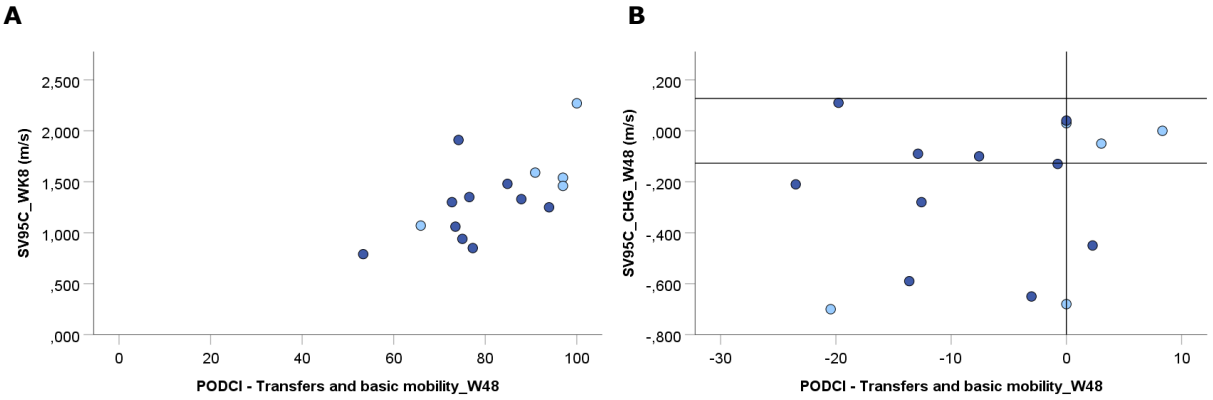


CGI-C = Clinical Global Impression of Change; MDC = minimal detectable change; SV95C = 95th centile of the stride velocity

A) Correlation of SV95C vs the CGI-C, both measured at Week 48 (Spearman ρ=-0.816, P-value=0.001). B) SV95C change at Wk48 vs CGI-C, horizontal lines represent the positive and negative MDC80% (Spearman ρ=-0.395, P-value=0.204

Similarly, a correlation between the SV95C and the PODCI sub score corresponding to the PODCI subdomain "transfers and basic mobility" was observed (Spearman coefficient correlation ρ = 0.611, P-value = 0.015 at Week 48), but no correlation was found between the changes from Baseline. Nevertheless, except for one, every child of parents who reported a lower transfers and basic mobility score at Week 48 as compared with baseline experienced a decrease in SV95C (Figure 33).

Figure 33: Relationship at Week 48 Between SV95C and PODCI (Subdomain Transfers and Basic Mobility)



CGI-C = Clinical Global Impression of Change; MDC = minimal detectable change; PODCI = Pediatrics Outcomes Data Collection Instrument; SV95C = 95th centile of the stride velocity

A) Correlation between SV95C measured at Week 48 and the PODCI-Transfer and basic mobility sub score (Spearman ρ = 0.611, P-value = 0.015). B) Changes from Baseline, horizontal lines represent the positive and negative MDC80%

The results of the analyses to assess within group meaningful change based on the CGI-C and PODCI categories are presented in Table 68. Overall, patients who were categorized as improved based on the CGI-C (clinician response of either "very much improved," "much improved," or "minimally improved") had the smallest decrease in the median change of SV95C over 48 weeks (-0.025 m/s). No patients

were categorized as improved (defined as a ≥ 10% gain) based on the PODCI. Similarly, the largest decrease (indicating a greater worsening) in the SV95C was observed in patients who were categorized as worsened based on the CGI-C (clinician response of either "minimally worse," "much worse," and "very much worse") and the PODCI (≥ 10% loss; -0.280 m/s and -0.245 m/s, respectively).

Table 68: Summary of Within-group Meaningful Change for the SV95C at Week 48

	Number of Patients	Mean Change of SV95C Over 48 Weeks	Median Change of SV95C Over 48 Weeks
CGI-C			
Improved	N = 4 (4 minimally improved, 0 much improved, and 0 very much improved)	-0.175 m/s	-0.025 m/s
Stable	N = 5	-0.302 m/s	-0.210 m/s
Worsened	N = 3 (0 minimally worse, 3 much worse, 0 very much worse)	-0.370 m/s	-0,280 m/s
PODCI			
Improved (≥ 10% gain)	N = 0	N/A	N/A
Stable	N = 9	-0.221 m/s	-0.100 m/s
Worsened (≥ 10% loss)	N = 6	-0.293 m/s	-0.245 m/s

CGI-C = Clinical Global Impression of Change; PODCI = Pediatrics Outcomes Data Collection Instrument; SV95C = 95th centile of the stride velocity

Despite the absence of efficacy of the investigational medicinal product, the large majority reported a minimal improvement or a stabilization and only 3 patients were reported with a worse clinical global impression of change after 48 weeks of follow up. While no improvement was reported by the patients/parents on the PODCI, a negative change in the PODCI or CGI-C reported by the clinicians is likely to be associated with an important change in the clinical state of the child and therefore would correspond to a specific milestone in the course of the disease (such as the loss of running ability or the inability to climb stairs), and thus could be used to identify meaningful change. In those patients who had been reported to be stable or worsened, the median SV95C change was between -0.100 and -0.280 m/s after 48 weeks of follow up. Nevertheless, the difference between the median SV95C change to consider a subject stable or worsened was -0.145 m/s and -0.070 m/s considering PODCI and CGI-C respectively. On this basis and considering that some worsened patients would have a smaller change than the overall median change, an anchor based MCT of around -0.1 m/s could be taken to indicate a meaningful change.

Anchor based on traditional endpoints

The SV95C MCT was also assessed through an anchor-based approach using the change over 48 weeks of reference functional tests performed in the CT-B study, namely 6MWD (N=13) and NSAA (N=12). The results of the analyses to assess within group meaningful change based on the $MDC_{80\%}$ of NSAA

(2.78) and 6MWD (36.3m) are presented in (Table 69). Patients who categorized as worsened based on median NSAA change from -2 to -3 points after 48 weeks had a median SV95C change of -0.115m/s (N=4). Similarly, patients categorized as worsened based on median 6MWD change from -30 to -40 m had a median SV95C change of -0.130m/s (N=3).

This method also supports an anchor-based MCT around 0.1 m/s.

Table 69: Summary of Within-group Meaningful Change for the SV95C at Week 48 based on standard functional tests

	Number of Patients	Mean Change of SV95C Over 48 Weeks	Median Change of SV95C Over 48 Weeks
NSAA			
Improved - [2 ; 3]	N = 1		
Stable -]2 ; 2[	N = 7	-0.246 m/s	-0.090 m/s
Worsened – [-2 ; -3]	N = 4	-0.228 m/s	-0,115 m/s
6MWD			
Improved - [30 ; 40]	N = 1		
Stable -]-30 ; 30[	N = 9	-0.140 m/s	-0.090 m/s
Worsened – [-40 ; -30]	N = 3	-0.287 m/s	-0.130 m/s

6MWD = 6-minute walk distance, NSAA = North Star Ambulatory Assessment, SV95C = 95th centile of the stride velocity

3.2.2.5.3. Overall estimate of MCT for the SV95C

The results of the distribution- and anchor-based analyses of meaningful change suggest that a change score of at least ≈-0.10 m/s would be required for the change in DMD patients to be beyond measurement error evaluated at 0.07 m/s, and that a change score of between -0.10 and -0.20 m/s would be meaningful.

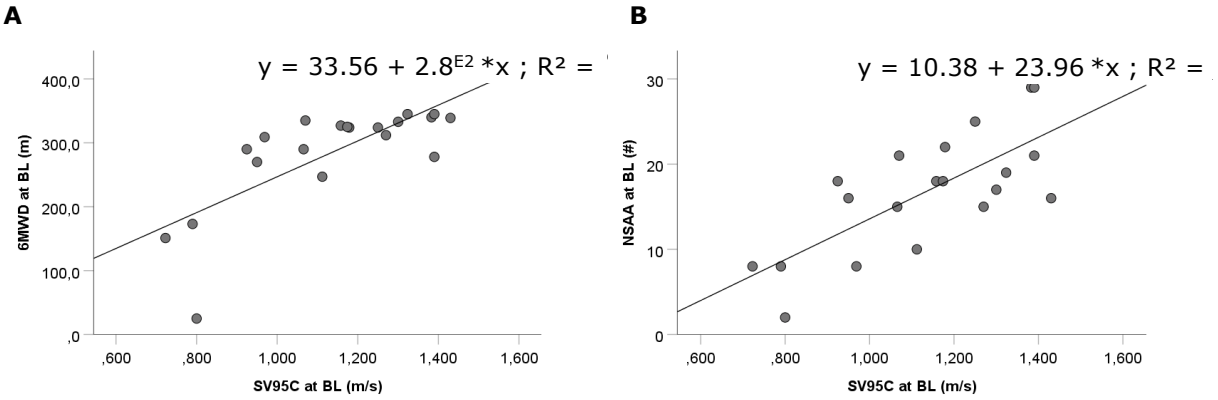
Interestingly, transposing into the natural course of the disease and steroids initiation, those thresholds were reached after 6, 9, and 12 months of follow up in the untreated population (Median changes from baseline scores were -0,07, -0,11, and -0.20 m/s respectively) (section 3.2.2.4.2) and as early as 3 months in patients starting corticosteroids, (median SV95C changes were 0.09 and 0.211 m/s at 3 and 6 months respectively) (section 3.2.2.4.4). Decline observed at 9 and 12 months was confirmed with median changes in 6MWD and NSAA from baseline (median 6MWD changes were -36.3m and -31.5 m and median NSAA change were -1 and -2 at 9 and 12 months respectively). The short-term efficacy of steroids leading to a global clinical improvement of DMD patients as early as 3 months after corticoids initiation, has been published for a while,[41,42] therefore suggesting that a median SV95C change of 0.09 m/s, beyond the measurement error could be a relevant MCT.

Overall, taking an estimate of **0.1 m/s for MCT** would be reasonable This value is larger than the estimate of measurement error and is consistent with the anchor-based change score in those patients considered to have worsened (PRO and reference functional tests) as well as change score in patients

who improved after the initiation of the steroids.

In addition, a change of 0.1 m/s in stride velocity implies a change in the distance walked in 6 minutes of 36 m, corresponding to the meaningful change threshold recognized in 6MWD for the disease course of DMD[43,44]. Finally, considering weaker patients with DMD (6MWD < 350 m and SV95C<1.50m/s), where a linear regression with a $R^2 > 0.5$ can be modelized, a change of 0.1 m/s in SV95C corresponds respectively to a change of 28 m in 6MWD and of 2.4 points in NSAA total score which were considered as a clinically change by the community (Figure 34).

Figure 34: Linear regressions between 6MWD (A) or NSAA (B) and SV95C for patients with 6MWD < 350 m and SV95C < 1.500 m/s



6MWD = 6-minute walk distance, NSAA = North Star Ambulatory Assessment, SV95C = 95th centile of the stride velocity

Anchor-based data from ongoing studies may further confirmed this threshold.

4. Generalization to Other Progressive NMDs with Proximal Muscle Weakness

There are other progressive NMDs characterized by proximal muscle weakness such as SMA, CNM, LGMD, or FSHD in which progressive loss of ambulation is the final stage of progressive walking difficulties. The SV95C is therefore also potentially a relevant outcome for these populations. Both qualitative and quantitative evidences were generated from these other proximal NMDs. They show consistent findings compared to those presented for the DMD population. Therefore, it demonstrates its interest in those conditions and further solidifies confidence in the interpretation of data in DMD.

As stated for DMD in Section 2.1, measuring disease progression and response to treatment in progressive NMDs characterized by a proximal muscle weakness is a challenge for all clinical development plans. This translates in the need of larger cohort and longer clinical trials, which is difficult or impossible to achieve in the context of very rare diseases. SV95C is therefore an appropriate outcome to use in clinical trials that aim to demonstrate the efficacy of a treatment in maintaining, improving, or reducing the decrease of the walk ability of such NMD patients, or in natural history studies that aim to characterize the course of the disease. In contrary to FSHD or CNM, a few treatments have been recently approved in SMA (e.g., Evrysdi®, Zolgensma®, Spinraza®) but longitudinal follow up of drug efficacy is still under investigation.

As for DMD, patients with proximal NMDs leading to walking disability, the maximal stride velocity decreases over time. Any stabilization or improvement in this maximal stride velocity would thus be indicative of an improvement or delay in progression of the disease. SV95C could therefore be used to assess the change in the stride velocity induced by an investigational medical product. SV95C may be also used to assess the change from baseline in stride velocity over time during the natural course of the disease, which could be used as part of a broader measurement strategy.

As for DMD, the targeted population to use SV95C as an endpoint in clinical trials includes ambulant patients genetically diagnosed with progressive NMD characterized by a proximal muscle weakness (ambulant meaning able to walk 10 steps [5 strides] independently). This includes LGMD, BMD, SMA types 3 and 4, FSHD, and CNM.

LGMD encompasses a large group of genetic disorders (over 40 genes), recessive or dominant, that share as a main phenotypic trait a predominantly progressive proximal weakness. These conditions may start in the infancy or in the adulthood. Most of them lead ultimately to loss of ambulation within 10 to 30 years after symptoms onset. The most commons of these condition are LGMD2A (calpainopthy), LGMD2I (related to FKRP) or LGMD2E (dysferlinopathy). Several preclinical or early clinical development are currently ongoing. Interestingly, BMD, commonly considered as a "milder" form of DMD and caused by an in-frame mutation of the *dystrophin* (and thus to the production of a truncated dystrophin) is considered as a LGMD2I.[45]

SMA is a recessive condition that affects 1/10.000 newborns. In about 15% of cases, patients may acquire the ability to walk, and will present the first difficulties after the age of 18 months, which define the SMA Type 3 phenotype. A very small percentage of patients experience symptoms onset in the adulthood and are classified as SMA type 4. Patients with SMA Type 3 experience progressive walking difficulties, eventually leading to loss of ambulation in about 60% of these patients. These difficulties are mostly due to the weakness of lower limb girdle. Interestingly, 3 drugs are currently approved in these conditions, even where no trial was conducted in SMA Type 3 for any of these drugs, mostly because of the impossibility to power appropriately a clinical trial in this rare and slowly progressive condition.[46]

FSHD is caused by the inappropriate expression of a junk gene- DUX4, that can be caused by different mechanisms. FSHD is a dominant disorder, but neo mutation are common. Patients may present with several pattern of weakness, but a certain percentage of them present with a proximal weakness leading to ambulation difficulties and loss of ambulation. This is true especially for the infantile form that ultimately always leads to loss of ambulation. SV95C has been used in one of the first international multicenter international trial[47] and has demonstrated high sensitivity to change in comparison with other measures. Several clinical trials are currently ongoing and face the same challenge of selecting a proper primary outcome.

CNM constitutes an heterogenous group of congenital myopathy that share the common pathological trait of a largely increased number of internalized nucleus in muscle fibers. CNM may be due to several genes mutations which translates into different transmission mode (X linked, dominant, recessive, sporadic).[48] Several clinical development are currently ongoing in these conditions.

The spectrum of genetic neuromuscular conditions is much broader. It includes other very rare proximal conditions, including metabolic or mitochondrial myopathy. In addition, muscle diseases may also present with mostly distal myopathy, such as MD or some form of distal myopathy.

The present application covers the conditions that are causing mostly proximal weakness leading to difficulties in ambulation. It does not cover conditions that do not present this component of significant proximal lower limb weakness.

The device used to record SV95C in patients from 5 years of age can technically be used in younger patients as long as they accept to wear the device long enough to get a sufficient amount of data (i.e., 50 hours, based on DMD) to compute accurate variables. The device is also considered to be suitable for use by patients from any country. With SV95C being a digital COA collected in a real-life setting, there are no foreseen limitations due to language or culture.

4.1. Methods

4.1.1. Qualitative Evidence (Content Validity)

To determine the clinical relevance of passively collecting the maximal ambulation speed in an uncontrolled environment to assess the efficacy of a new drug in other NMDs, information was obtained from patients and caregivers across a number of NMDs via the online survey discussed in Section 3.1.1. The survey was completed by a total of 549 patients and caregivers. In addition to the 92 DMD patients and caregivers already discussed, this included 457 living with, or caring for someone with, other NMDs, such as LGMD (n = 116; 102 patients and 14 caregivers), MD (n = 105; 96 patients and 9 caregivers), FSHD (n = 128; 120 patients and 8 caregivers), SMA (n = 47; 31 patients and 16 caregivers), CNM/MTM (n = 43; 26 patients and 17 caregivers), and others who listed their condition as "other" (n = 18; 14 patients and 4 caregivers). A copy of a report from the survey is provided in Section 7.2). Data from the 334 respondents either living with, or caring for someone with, other NMDs that are progressive with proximal muscle weakness (akin to DMD) are presented. Data from those with MD and who indicated "other NMD" in the survey are presented in the survey report (Section7.2) but not within this dossier.

The objectives of the patient/caregiver survey were:

- To collect what was important to them in terms of ambulation.
- To determine what were their first symptoms, how the disease impacts their mobility and their family activities, which functions they would like to see maintained, improved, or restored by a treatment, what they consider as a clinical change in terms of ambulation improvement, and if they would accept wearing a wearable device at home to monitor their walking abilities.

4.1.2. Quantitative Evidence

Additional evidence from other NMDs is also provided to support the primary endpoint application in patients with DMD. Based on the clinical trials currently ongoing, the following clinical properties in were also tested on patients living with SMA, CNM, FSHD, and LGMD. Data were collected from a total of 52 patients (20 patients with SMA [from Studies NatHis-SMA and Acti-SMA], 8 patients with CNM [from Study NatHis-CNM], 19 patients with FSHD [from Studies aTyr-C004 and FIS-001-2019], and 5 patients with LGMD (from Study aTyr-C004), along with 93 healthy control subjects without any muscular condition (refer to Table 82 for additional details).

4.1.2.1. Accuracy

The degree to which the measure assesses with the wearables is intended to measure has been evaluated in 4 patients with SMA and 2 patients with CNM by comparing their walked distance in 6 minutes measured with physiotherapists during the 6-minute walking test and the distance calculated with ActiMyo®. 7 and 2 tests have been analyzed respectively (Table 70).

Table 70: List of Subjects Used for Accuracy Assessment

Group	Patient ID
SMA	246, 248, 249, 252
CNM	259, 264

CNM = centronuclear myopathy; SMA = spinal muscular atrophy

4.1.2.2. Repeatability

4.1.2.2.1. Test-retest Reliability

Test-retest reliability consists of measuring the degree to which a device measures the outcome the same way at 2 points in time, under the same assessment condition. Test-retest reliability was assessed by calculating an ICC coefficient in 6 patients with SMA, 4 patients with CNM, and 14 patients with FSHD from NHS-CNM, NHS-SMA-A and CT-FSHD-B studies respectively, with measures performed 1 month apart in 2 successive recording periods (Table 71). Results were further supported by Bland-Altman plots.[33-35]

Table 71: List of Subjects Used for Test-retest Reliability Assessment

Group	Patient ID
SMA	236, 237, 238, 239, 241, 242
CNM	259, 260, 261, 265
FSHD	273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286

CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; SMA = spinal muscular atrophy

4.1.2.2.2. Robustness

Robustness refers to the degree to which a system continues to function in the presence of invalid inputs or stressful environmental conditions.

In the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019, we detailed the intra-patient variability analysis on 28 DMD patients who have worn ActiMyo for at least 1800 hours. This analysis demonstrates the low variability of the SV95C calculated over a period of 180 hour (4.41%). During this intra-patient analysis, we determined that over 50 hours of recorded data, the intra-patient variability found for the SV95C is 6.38%, which was acceptable as compared with the 6MWD test, which has a variability around 10%. Below 50 hours of recording, the data suggested the variability increases exponentially.

To verify those thresholds on other NMD, the relation between the recording period duration and the variability of the measurement was studied with the adjusted Variance of Allan (Sysnav Variance) in 4 patients with SMA and 3 patients with CNM with at least 1800 hours of recording (Table 72).

Table 72: Lists of Patients Used to Verify the Acceptability of Thresholds of Recording Period Duration

Group	Patient ID
SMA	236, 237, 241, 242
CNM	259, 263, 265

CNM = centronuclear myopathy; SMA = spinal muscular atrophy

The influence of the time of recording (e.g., morning versus afternoon and weekday or weekend), on SV95C variability was also assessed in all patients enrolled in the SMA, CNM, FSHD, and LGMD populations (Table 73).

Table 73: List of Patients with SMA, CNM, FSHD, and LGMD

Group	Patient ID
SMA	236, 237, 238, 239, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 254, 255, 256, 257
CNM	258, 259, 260, 261, 262, 263, 265
FSHD	267, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286
LGMD	287, 290, 292, 293, 294

CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = Limb girdle muscular dystrophy; SMA = Spinal muscular atrophy

4.1.2.3. Construct Validity

4.1.2.3.1. Known-groups Validity

To confirm the clinical validity of the SV95C in the other NMD populations, all patients with SMA, CNM, FSHD, and LGMD (Table 73) were compared with healthy control subjects without any muscle condition (Table 74) and to patients with DMD (Table 6) using the independent samples Kruskal-Wallis test (Limit of statistical significance = 0.05). When statistical significance was found, post hoc analysis was performed, i.e., pairwise comparisons for each condition, with significance values adjusted by the Bonferroni correction for multiple tests. Additional subgroup analyses included patients stratified by age range (5 to 17 years and 18 to 84 years).

Table 74: List of Control Subjects Used for the Known-groups Validity

Group	Patient ID
CTRL	136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 198, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235

CTRL = control population

4.1.2.3.2. Convergent Validity

The convergent validity of the SV95C was evaluated in patients with SMA, CNM, FSHD, and LGMD by cross-correlating SV95C with existing COAs (6MWD, NSAA, 4SC, MFM and Vignos when available; Table 75). Correlations between SV95C and the existing COAs were measured in a 1-month period around the first onsite visit (baseline visit). Patients with DMD and healthy subjects without any muscle conditions were displayed graphically.

Table 75: List of Patients with SMA, CNM, FSHD, and LGMD Used for Assessment of Convergent Validity

Pathology	Patient ID
SMA	236, 237, 238, 239, 241, 242, 246, 247, 248, 249, 250, 251, 255, 256, 257
CNM	258, 259, 260, 261, 262, 263, 265
FSHD	273, 274, 275, 276, 277, 278, 279, 280, 282, 283, 284, 285, 286 267, 269, 270, 271, 272 (correlation with Vignos scale only)
LGMD	287, 290, 292, 293, 294 (correlation with Vignos scale only)

CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = Limb girdle muscular dystrophy; SMA = spinal muscular atrophy

4.1.2.4. Responsiveness (Ability to Detect Change)

The sensitivity to change of the SV95C was assessed by studying the natural change of the SV95C over time at 3, 6, 9, and 12 months measured in a limited group of patients with SMA (N = 5, 6, 4, 3, respectively) and CNM (N = 7, 6, 3, 3). Changes were assessed with a one-sample Wilcoxon rank test with the null hypothesis being that the median change is zero. In addition, responsiveness was assessed in SMA patients treated with Spinraza (nusinersen) (N = 11, 7, 7, 5 respectively at 3, 6, 9 and 12 months of follow up; Table 76).

Table 76: List of Patients with SMA, CNM, FSHD, and LGMD Used for Studying *Responsiveness*

Timepoint	Patient ID	
	Natural Course of the Disease	Treatment Effect
SMA		
3 months FU	236, 238, 239, 241, 242	245, 246, 247, 248, 250, 251, 252, 254, 255, 256
6 months FU	236, 237, 238, 239, 241, 242	245, 246, 248, 249, 250, 251, 252, 254, 255, 256, 257
9 months FU	237, 239, 241, 242	245, 246, 247, 249, 251, 252, 254, 255, 256, 257
12 months FU	237, 241, 242	246, 247, 248, 249, 250, 251, 252, 256, 257
CNM		
3 months FU	258, 259, 260, 261, 263, 265	
6 months FU	258, 259, 260, 261, 263, 265	
9 months FU	259, 263, 265	
12 months FU	259, 263, 265	

CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; FU = follow up; LGMD = limb girdle muscular dystrophy; SMA = spinal muscular atrophy

4.1.2.5. Meaningful Change Thresholds - Distribution-based

The SEM was calculated using the formula SEM = SD*SQR(1-ICC) wherein the ICC was calculated based on the specifications provided in Section 3.1.2.2. A related concept, the MDC, was calculated based on the formula MDC = z-score*SEM*SQR(2), with Z-scores equal to 1.960, 1.645, and 1.282 at 95%, 90%, 80% confidence levels, respectively. Patients used to calculate MCT based on the data distribution are listed in Table 77.

Table 77: List of Patients with SMA, CNM, FSHD, and LGMD Used to Determine MCT Based on the Data Distribution

Pathology	Patient ID
SMA	236, 237, 238, 239, 241, 242, 246, 247, 248, 249, 250, 251, 252, 256, 257, 254, 255, 243, 244
CNM	258, 259, 260, 261, 263, 265
FSHD	273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 267, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286
LGMD	287, 290, 292, 294

CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; MCT = meaningful change threshold; SMA = spinal muscular atrophy

4.2. Results

4.2.1. Qualitative Evidence (Content Validity)

Patient and Caregiver Online Survey

A total of 549 respondents (403 patients and 146 caregivers) participated in the survey, the full data related to ambulation from this survey can be found in Section 7.2. However key findings from the 334 respondents which represented patients with progressive NMDs with proximal muscle weakness are presenting in this section. This included FSHD (n = 128; patients = 120; caregivers = 8), LGMD (n = 116; patients = 102; caregivers = 14), SM (n = 47; patients = 31; caregivers = 16), and CNM/MTM (n = 43; patients = 26; caregivers = 17).

Overall, these results confirm that ambulation is a key aspect of all the other progressive NMD conditions captured, with walking playing a key part especially for those who are ambulant, and the benefits of using a wearable device in a real-life setting to capture mobility in clinical trials being recognized.

Table 78 summarizes the key characteristics of respondents for each selected other progressive NMD populations that completed the survey. The severity of progressive NMDs with proximal muscle weakness varied for each condition. Patients with NMDs diagnosed during childhood were mostly non-ambulant, while patients with NMDs diagnosed during adulthood were mostly ambulant. In addition, reports from NMDs diagnosed during childhood were mostly completed by caregivers while reports from NMDs diagnosed during adulthood were mostly completed by patients themselves.

Table 78: Characteristics of the Survey Population – Other Progressive NMDs

		SMA (n = 47)	CNM/MTM (n = 43)	FSHD (n = 128)	LGMD (n = 116)
Age of patient (in years)	Mean (SD)	33.8 (21.62)	30.0 (23.29)	52.2 (15.90)	39.7 (17.03)
	Median	34.0	24.0	56.0	39.5
	Min – Max	2 – 81	1 – 76	11 – 84	2 – 75
	Q1, Q3	14.0, 54.0	11.0, 49.0	41.0, 63.5	28.0, 52.0
Age symptoms first appeared (in years)	Mean (SD)	8.5 (13.96)	7.6 (14.55)	23.8 (17.01)	16.4 (13.52)
	Median	3.0	0.0	18.0	11.0
	Min – Max	0 – 60	0 – 50	0 – 90	0 – 65
	Q1, Q3	1.0, 11.0	0.0, 10.0	12.0, 32.5	7.0, 25.0
	Missing	5	2	0	5
Ambulant[1]	Yes	6 (12.8%)	11 (25.6%)	78 (60.9%)	62 (53.4%)
	No	40 (85.1%)	30 (69.8%)	49 (38.3%)	53 (45.7%)
	Prefer not to respond	1 (2.1%)	2 (4.7%)	1 (0.8%)	1 (0.9%)
	Missing	0	0	0	0
Relationship to patient	Caregiver	2 (12.5%)	0	0	0
	Father	4 (25.0%)	2 (11.8%)	0	5 (35.7%)
	Mother	10 (62.5%)	14 (82.4%)	5 (62.5%)	7 (50.0%)
	Grandparents	0	0	0	0
	Legal guardian	0	0	0	0
	Life partner	0	1 (5.9%)	1 (12.5%)	1 (7.1%)
	Sibling	0	0	0	0
	Other	0	0	2 (25.0%)	0
	Prefer not to respond	0	0	0	1 (7.1%)
	Missing[2]	31	26	120	102

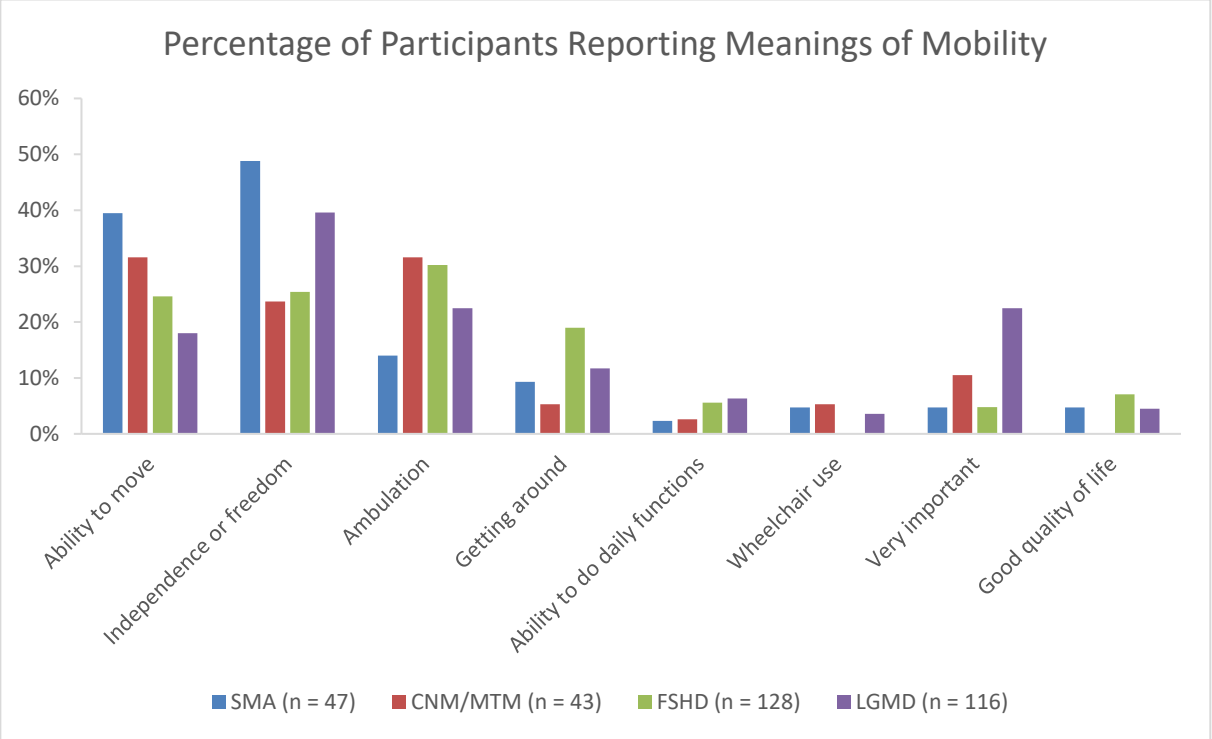
CNM/MTM = centronuclear and myotubular myopathy; FSHD = facioscapulohumeral dystrophy; LGMD = limb girdle muscular dystrophy; MD = myotonic dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; Max = maximum; Min = minimum; N = number of subjects in the population; Q1 = first quartile (25th percentile); Q3 = third quartile (75th percentile); SD = standard deviation

[1]Ambulant defined as ability to walk 10m (25ft) without help, based on survey response.

[2]Question not answered by patients as respondents, only by caregivers.

When asked to describe the meaning of mobility for them, the responses were similar across the other progressive NMD conditions and consistent with those with DMD population reported in the previous section – an ability to move, getting around, and independence or freedom. Ambulation was related mostly to walking but also running and climbing stairs. Some participants simply indicated that mobility was "very important" and linked to good quality of life (see Figure 35).

Figure 35: Meaning of Mobility in SMA, CNM, FSHD, and LGMD Populations



CNM = centronuclear myopathy; CNM/MTM = centronuclear and myotubular myopathy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; SMA = spinal muscular atrophy

Responses shown for those meanings indicated by 9 or more participants within a condition.

Note: A total of 4 participants were considered missing in the SMA group, n = 5 in the CNM/MTM group, n = 2 in the FSHD group, and n = 5 in the LGMD group.

Although there are differences between the progressive NMD conditions in precisely how and when ambulation is affected, when asked to describe the impact of the disease upon mobility, the responses were broadly similar across other NMD conditions and to those reported in DMD (Table 79) – walking, a reliance on technology and reliance on others being key themes. Similarly, when asked about the impact of the disease on family activities, ambulation was a key part of this for those with other NMDs, with most respondents in all groups reporting activities being limited (reported by 25% to 41%) and the need to plan ahead (reported by 8% to 24%) as having the most impact.

Table 79: Impact on Mobility by Disease (Non-DMD Only)

Impact on Mobility[1]	SMA (n = 47)[2]	FSHD (n = 128)[2]	CNM/MTM (n = 43)[2]	LGMD (n = 116)[2]
Missing	4	0	3	5
Walking impacted	13 (30.2%)	53 (41.4%)	13 (32.5%)	30 (27.0%)

Impact on Mobility[1]	SMA (n = 47)[2]	FSHD (n = 128)[2]	CNM/MTM (n = 43)[2]	LGMD (n = 116)[2]
Reliance on technology	19 (44.2%)	17 (13.3%)	8 (20.0%)	21 (18.9%)
Reliance on others	4 (9.3%)	7 (5.5%)	8 (20.0%)	20 (18.0%)
Weakness	3 (7.0%)	9 (7.0%)	3 (7.5%)	4 (3.6%)
Upper body mobility or strength	3 (7.0%)	11 (8.6%)	3 (7.5%)	7 (6.3%)
Social impacts	0	4 (3.1%)	0	6 (5.4%)
Household duties or everyday tasks	1 (2.3%)	8 (6.3%)	3 (7.5%)	8 (7.2%)
Limits physical activities	2 (4.7%)	12 (9.4%)	0	11 (9.9%)
No Impact	0	4 (3.1%)	0	3 (2.7%)
Falling or fear of falling	3 (7.0%)	9 (7.0%)	1 (2.5%)	5 (4.5%)
Decline in mobility	5 (11.6%)	5 (3.9%)	4 (10.0%)	7 (6.3%)
Fatigue	1 (2.3%)	7 (5.5%)	3 (7.5%)	4 (3.6%)
Large impact	1 (2.3%)	8 (6.3%)	4 (10.0%)	18 (16.2%)
Stairs difficult	1 (2.3%)	6 (4.7%)	5 (12.5%)	12 (10.8%)
Ability to stand	3 (7.0%)	5 (3.9%)	3 (7.5%)	4 (3.6%)
Pain	1 (2.3%)	15 (11.7%)	2 (5.0%)	3 (2.7%)
Balance poor	0	12 (9.4%)	1 (2.5%)	2 (1.8%)
Sitting ability	1 (2.3%)	2 (1.6%)	4 (10.0%)	2 (1.8%)
Some impact	0	1 (0.8%)	0	4 (3.6%)
Work	0	4 (3.1%)	0	3 (2.7%)

CNM/MTM = centronuclear and myotubular myopathy; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral dystrophy; LGMD = limb girdle muscular dystrophy; MD = myotonic dystrophy; SMA = spinal muscular atrophy

[1]Participants' responses could include multiple impacts. Impacts are not mutually exclusive, so column percentages may total more than 100%.

[2]Percentages reported are calculated based on the number of non-missing participants in the column

Despite slight differences between conditions, the following data are presented for all NMDs in the survey, except those with DMD (non-DMD respondents), including patients with MD [n = 105], and other unspecified NMDs [N = 18]). Further analyses per conditions will be performed.

When asked to rate the importance of various aspects of ambulation, as with DMD, the vast majority (85%) of the non-DMD respondents reported walking ability to be "very important." Self-transfer and limiting falls were also both frequently reported to be "very important" (80% for both). Walking was

important to both the non-ambulant and ambulant populations. Figure 36 and Figure 37.

Figure 36: Aspects of Ambulation – Importance Ratings of non-DMD Respondents

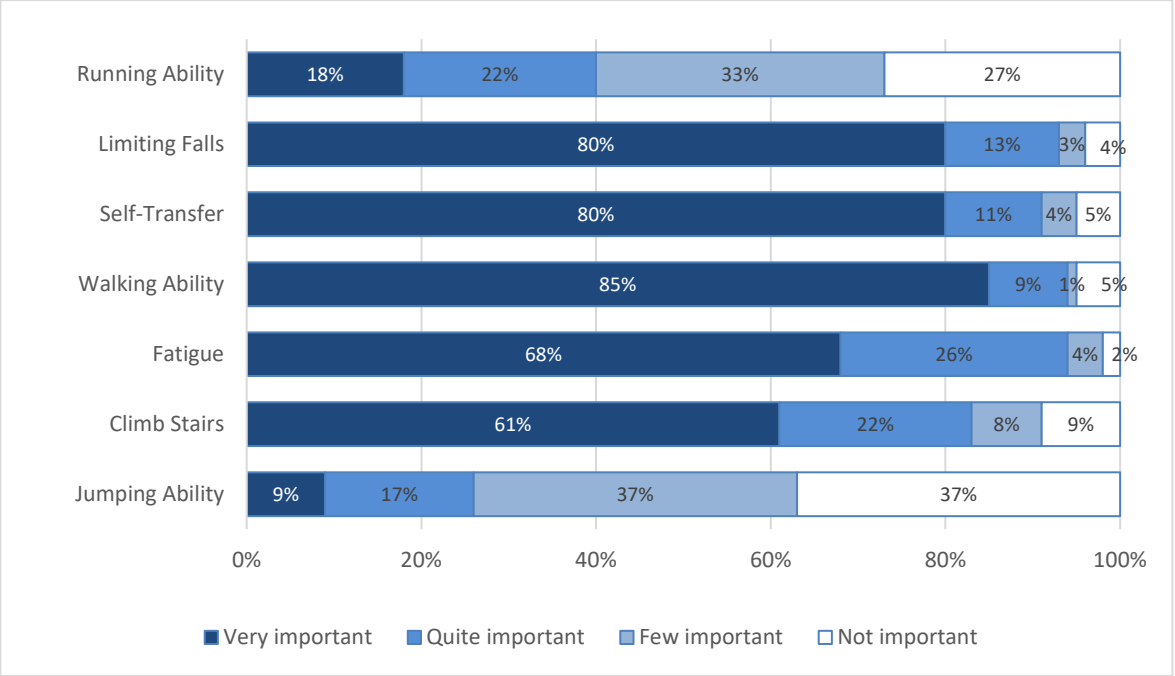
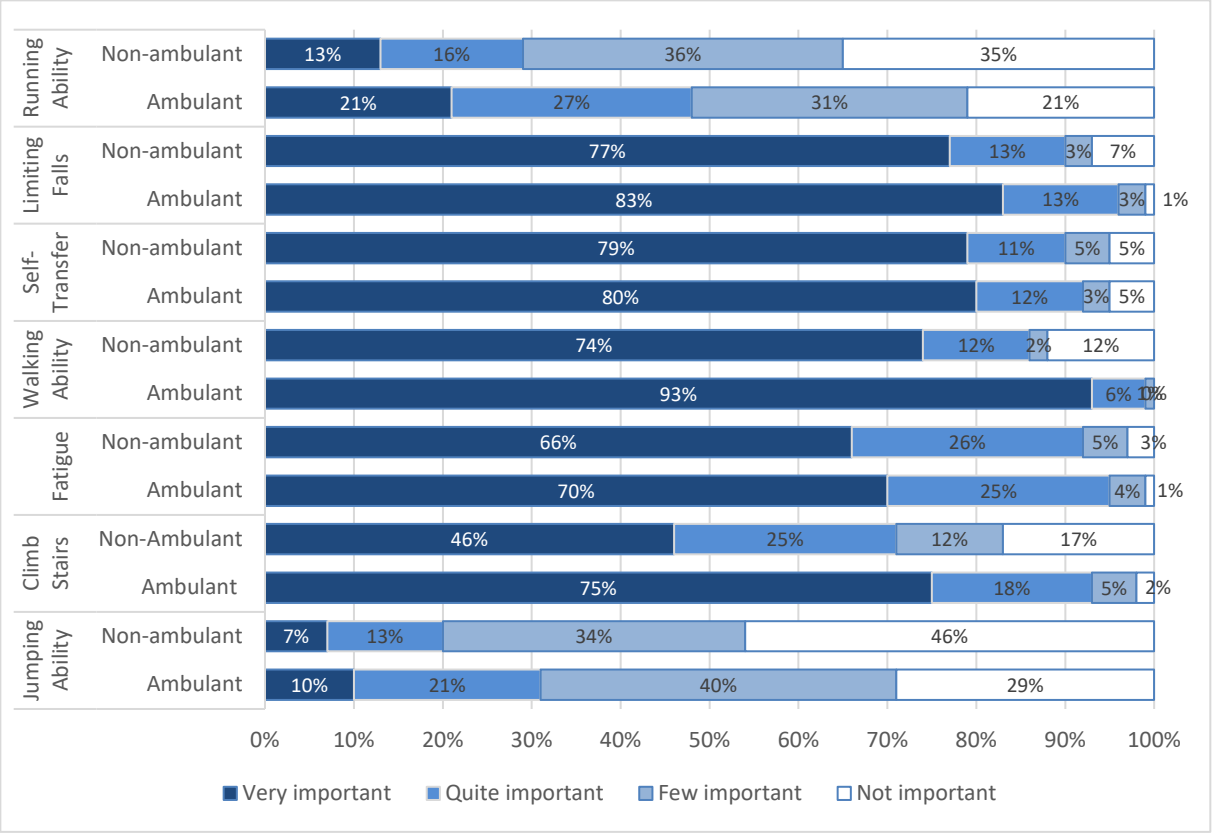


Figure 37: Aspects of Ambulation – Importance Ratings of non-DMD Respondents by Walking Ability



When asked in the context of a clinical trial, ambulation and mobility, along with muscle strength, were

identified as key functions to maintain for all non-DMD conditions captured in the survey, except SMA. In SMA the focus was on muscle strength. However, when asked about functions to improve or restore, ambulation and mobility, along with muscle strength, were key for all non-DMD conditions, including SMA (see the survey report for more details).

When asked about aspects of ambulation that best represent improvement, the non-DMD population most commonly reported outcomes relating to the distance walked (either before stopping or per day; n = 184 in total out of those who answered these questions). Fatigue during ambulation was also commonly reported. See Table 80 for results for the non-DMD survey respondents combined. These results indicate that walking speed is relevant to these NMDs as it is with DMD, which was confirmed by 74% of those asked in the non-DMD population indicating that a change in top walking speed would represent an improvement in ambulation. This was reflected across both the ambulant and non-ambulant patients. Also, as with DMD, a change in approximately 20 to 40 meters was considered by most (70%) to be an improvement.

Table 80: Aspects of Ambulation That Best Represent Improvement in the non-DMD Respondents

Best represents an ambulation improvement*	Non-DMD (n = 457)
Fatigue during ambulation	114 (24.9%)
Distance walked before stopping	101 (22.1%)
Number of falls per day	39 (8.5%)
Ability to climb stairs	90 (19.7%)
Ease in climbing stairs (both feet on each step or one foot per step)	61 (13.3%)
Distance walked per day	83 (9.0%)
Time measured to climb stairs	28 (6.1%)
Ability to walk fast	69 (15.1%)
Other	28 (6.1%)
Prefer not to respond	5 (1.1%)

DMD = Duchenne muscular dystrophy

When asked to provide specific feedback on use of a wearable device such as ActiMyo® in a clinical trial, 80% of non-DMD respondents indicated that they would prefer mobility to be assessed by a wearable device in a real-life setting than by regular clinic-based assessments by a physiotherapist or physician, and 72% felt that use of such a device would make taking part in the clinical trial more attractive. The majority (82%) also indicated that they would be willing to use ActiMyo®, most of

whom (64.7%) for as long as the trial lasts; see Table 81.

Table 81: Feedback on ActiMyo® Device in Non-DMD Respondents

Question	Response	Non-DMD (n = 457) n (%)
Prior use of ActiMyo®	No	403 (98.3%)
	Yes	6 (1.5%)
	Missing	47
Would device such as ActiMyo® make participating in clinical trials more attractive	No	83 (20.3%)
	Yes	297 (72.6%)
	I prefer not to respond	29 (7.1%)
	n missing	48
Willing to use ActiMyo®	No	8 (2.0%)
	Yes	339 (82.7%)
	I don't know	62 (15.1%)
	n missing	47
How long willing to wear ActiMyo®	As long as the trial lasts	224 (64.7%)
	2 weeks	15 (4.3%)
	1 month	24 (7.0%)
	6 months	14 (4.1%)
	1 year or more	6 (1.7%)
	I don't know	62 (17.9%)
	n missing	111
Most important limitation to wearing ActiMyo®	Tolerability / Discomfort of wearing the device	105 (25.9%)
	Size *and* weight of the device	19 (4.7%)
	The appearance of the device	46 (11.4%)
	The device is not waterproof	39 (9.6%)
	Duration of having to wear the device	11 (2.7%)
	Size *or* weight of the device	109 (26.9 %)
	Looking different because you are wearing the device	15 (3.7%)
	No limitation	57 (14.1%)

Question	Response	Non-DMD (n = 457) n (%)
	n missing	52

DMD = Duchenne muscular dystrophy

4.2.2. Quantitative Evidence

4.2.2.1. Population

Population characteristics and ActiMyo® configuration and recording periods used in each clinical study for the SMA, CNM, FSHD, and LGMD populations are listed in Table 2 and Table 82.

Table 82: Origin of Data Used in the Other Progressive NMD Populations

Study Reference Number	Pathology	Selection Criteria	N	ActiMyo® Configuration	Recording Periods
Natural history studies (NHS)					
NHS-SMA-A	SMA	[6 – 29] SMA type 2 and 3	6	Ankle / Wrist	Continuous recording up to 24 months
NHS-CNM-A	CNM	[6 – 62] MTM – DNM2 mutation	8	Ankle / Wrist	Continuous recording up to 24 months
Therapeutical studies (TTT)					
NHS-SMA-B	SMA	[4 – 43] SMA type 2 and 3	14	Ankle / Wrist	RP = 30 days RP every 4 months the first year then every 8 months
CT-FSHD-A	FSHD LGMD	[33 – 51] [27 – 39]	5 5	Ankle / Wrist	Continuous recording up to 4 months – Only BL data are used
CT-FSHD-B	FSHD	[23 – 58]	14	Ankle / Wrist	RP = 15 days RP every 15 days for 1 year – Only BL data are used

BL = Baseline; CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; RP = recording period; SMA = spinal muscular atrophy

4.2.2.2. Accuracy

The distance walked in 6 minutes measured by physiotherapists during the 6MWT and the distance calculated with the wearable device was compared in 4 patients with SMA (7 tests analyzed) and in 2 patients with CNM (2 tests analyzed). Results showed that the distances measured by physiotherapists

and those computed by wearable sensors were similar after adjusting for the distance from turning around the cones at each 25-meter corridor extremities in the 6MWT (see p25/77 of the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019). Differences were -1.4. ± 0.6 m for a mean distance walked in 6 minutes of 393.7 ± 72.4 m and -1.4 ± 2.5 m for a mean distance walked of 280 ± 225 m for SMA and CNM respectively (Table 83). ICC between 6MWD assessed by physiotherapists and ActiMyo® was 0.995 for patients with SMA traducing an excellent agreement between ActiMyo® and Physiotheraoists assessments (Figure 38).

This result suggests that ActiMyo® is able to detect strides of patients with other progressive NMDs characterized by a proximal muscle weakness even in very weak CNM and SMA patients walking only 120.5 or 183 m in 6 minutes respectively.

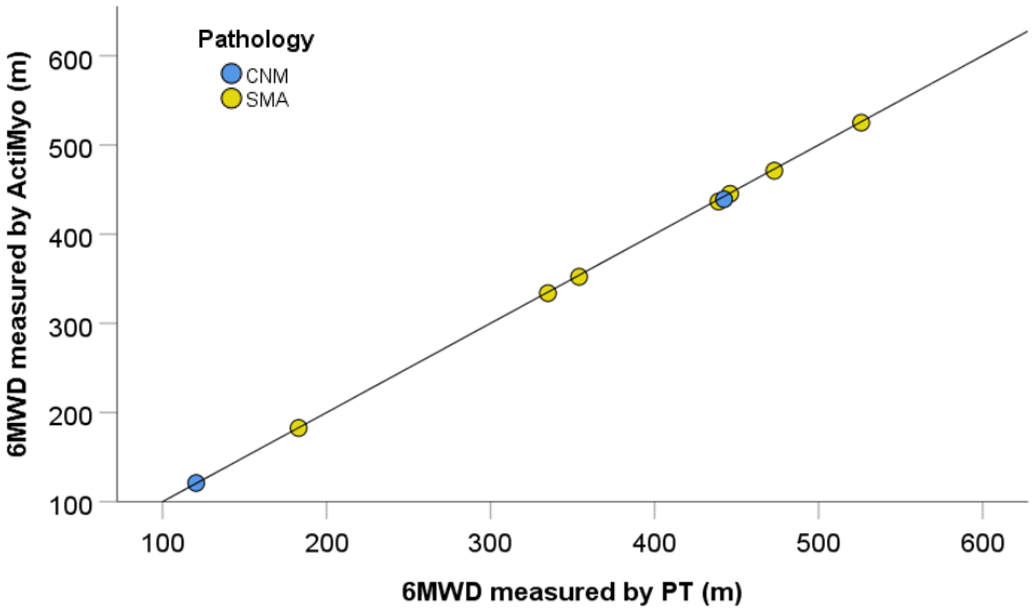
Table 83: Individual differences of the 6MWD measured by ActiMyo® as compared to 6MWD measured by physiotherapist

Patient_ID	Age at BL	Pathology	6MWD ActiMyo§	6MWD PT	6MWD difference PT-ActiMyo
246	25.2	SMA	445,2	446	-0,8
246	25.2	SMA	436,6	439	-2,4
248	10.1	SMA	471,1	473	-1,9
248	10.1	SMA	525	526	-1
249	47.7	SMA	333,8	335	-1,2
249	47.7	SMA	352,2	354	-1,8
252	51.5	SMA	182,6	183	-0,4
264	33.1	CNM	439,1	442,3	-3,2
259	6.6	CNM	120,9	120,5	0,4

6MWD = 6-minute walk distance; BL = Baseline; CNM = centronuclear myopathy; PT = Physiotherapist; SMA = spinal muscular atrophy

§ after the correction linked to the turn-distance

Figure 38: Comparison of the 6MWD measured by ActiMyo® to the 6MWD measured by a physiotherapist



6MWD = 6-minute walk distance; CNM = centronuclear myopathy; PT = Physiotherapist; SMA = spinal muscular atrophy

4.2.2.3. Repeatability

4.2.2.3.1. Test-retest Reliability

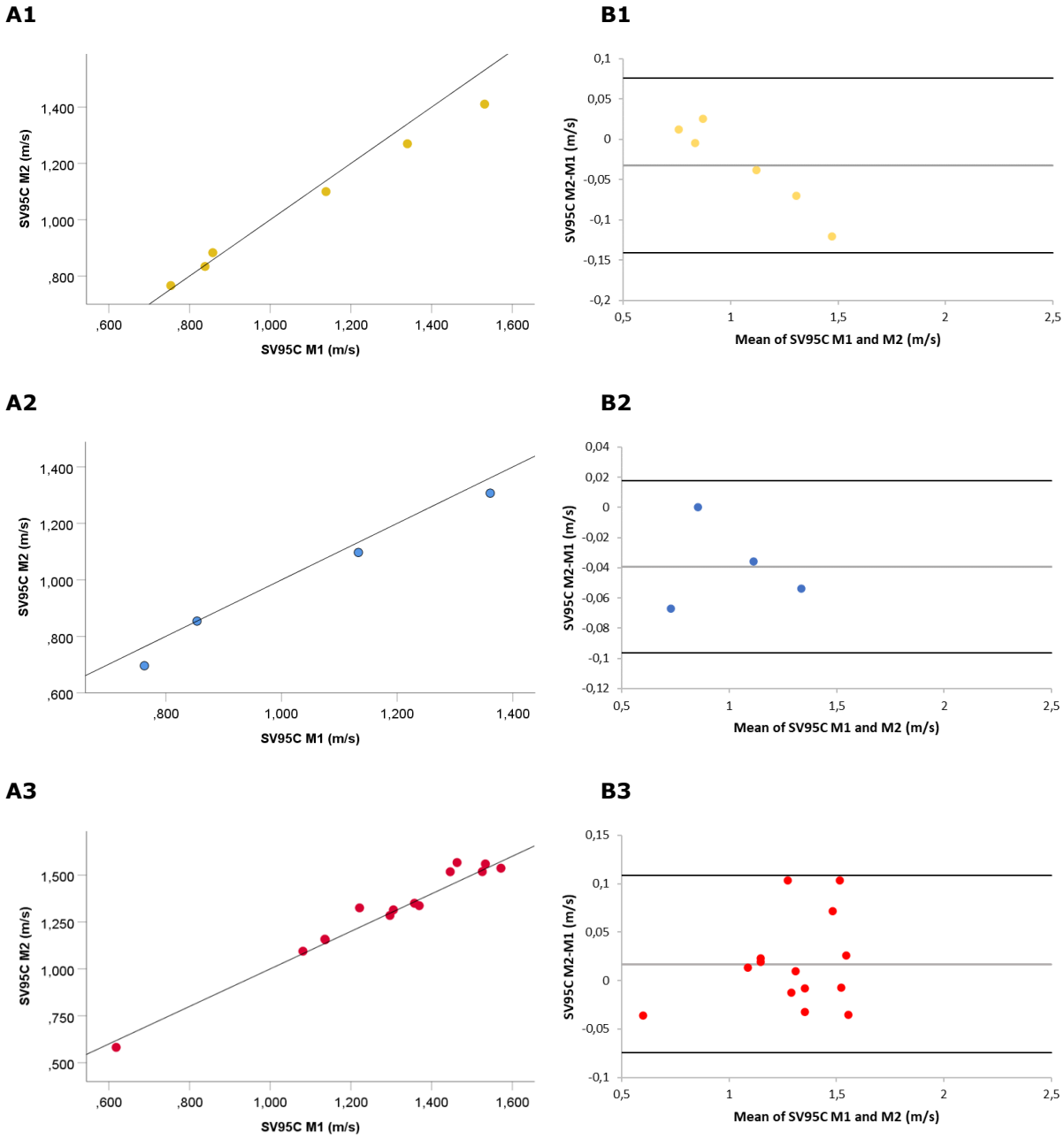
Based on test-retest reliability with measures performed 1 month apart in 2 successive recording periods for patients from Study NHS-SMA-A (N = 6), NHS-CNM-A (N = 6), and patients from Study CT-FSHD-B (N = 14; Baseline equaled 2 months), the ICCs in patients with SMA, CNM, and FSHD were high (0.999, 0.985, and 0.991, respectively), indicating excellent agreement between the 2 periods (Table 84 and Figure 39-A). These results were further supported based on Bland-Altman plots (Figure 39-B). While based on a small number of participants, these results confirm the acceptable reliability of SV95C.

Table 84: Test Retest reliability of the SV95C in Other Progressive NMDs

	N	ICC	95% CI
SMA	6	0.999	[0.927 – 0.998]
CNM	4	0.993	[0.717 – 1.000]
FSHD	14	0.991	[0.973 – 0.997]

CI = confidence interval; CNM = centronuclear myopathy; DMD = Duchenne muscular dystrophy FSHD = facioscapulohumeral muscular dystrophy; ICC = intra-class correlation coefficient; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Figure 39: Test-retest Reliability of the SV95C in SMA, CNM, and FSHD Populations



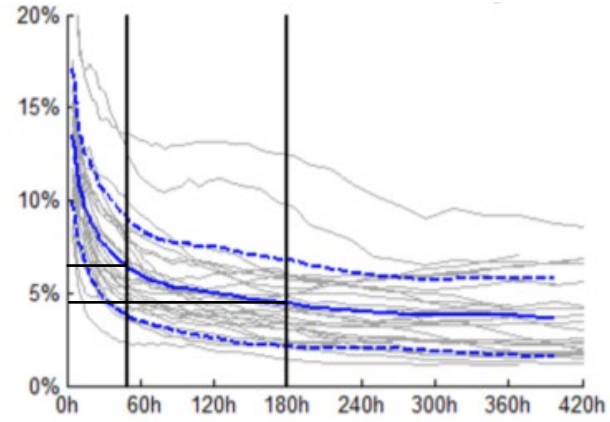
CNM = centronuclear myopathy; FSHD = facioscapulohumeral muscular dystrophy; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

A: Correlation between SV95C measured at month 1 (M1) vs month 2 (M2); B: Bland-Altman representation. A1-B1: SMA, A2-B2: CNM, A3-B3: FSHD;

4.2.2.3.2. Robustness

In the previous secondary endpoint qualification opinion package EMA/CHMP/SAWP/178058/2019, we demonstrated that SV95C should optimally be computed from 180 hours (with a minimum of 50 hours) of recording in a 2- to 4-week period. Over this time period, the patient's ambulation is not expected to change significantly given what is known about course of the DMD (see Figure 40).

Figure 40: Variability Plot for SV95C Versus the Number of Hours of Data



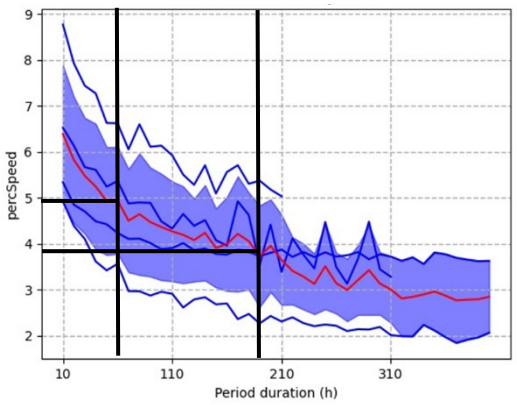
SD = standard deviation; SV95C = 95th centile of the stride velocity

Gray lines are data from individual patients. Blue line indicates mean curve and blue dashed line mean+/- SD.

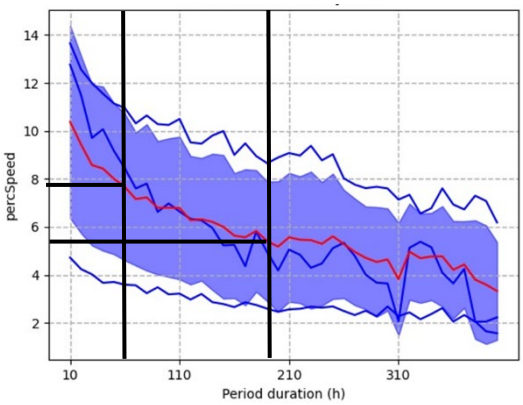
Using data from 4 SMA and 3 CNM patients assessed in a non-controlled setting, the relationship between the recording period average and the SV95C variability was assessed. Overall, a low variability about 4% and 5% reported for the SMA population (based on 180 and 50 hours of wearable device and system use, respectively). Similarly, for the CNM population, a recording period of 180 hours led to a variability of less than 6% and a recording period of 50 hours led to a variability about 8%
(Figure 41). Further investigations on larger sample sizes are necessary to confirm this observation.

Figure 41: Variability plot for SV95C Versus the Number of Hours of Data (SMA and CNM Populations)

A



B



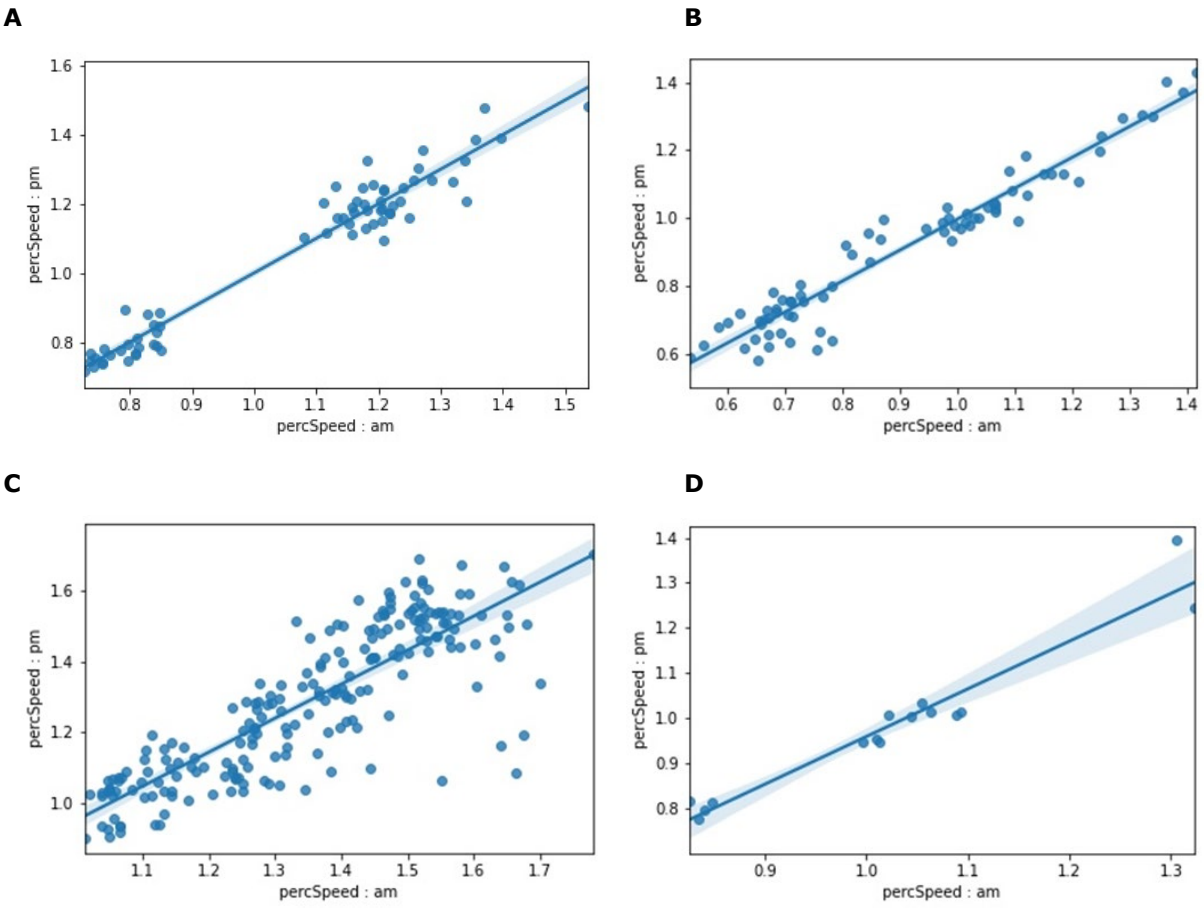
SD = standard deviation; SV95C = 95th centile of the stride velocity

A: SMA (Spinal muscular atrophy), B: CNM (centro-nuclear myopathy)

Blue lines are data from individual patients. Red line indicates mean curve and blue aera represents+/- SD.

Furthermore, there was no impact of recording in the morning (am) versus the evening (pm) for the SMA, CNM, LGMD, and FSHD populations, with the exception of some patients with FSHD (Figure 42), and no impact of recording during the week versus the weekend for any population (Figure 43).

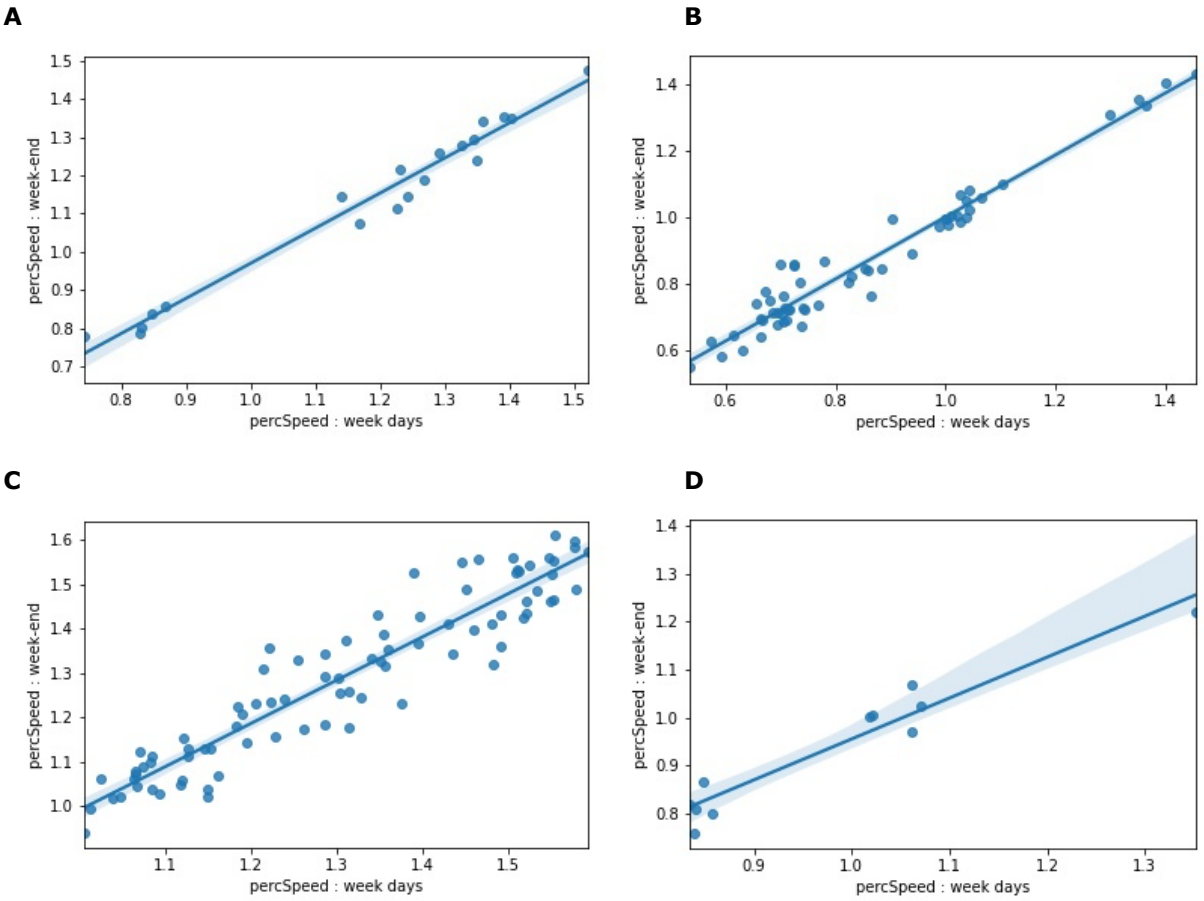
Figure 42: Comparison of SV95C Between Morning (AM) and Afternoon (PM)



SV95C = 95th centile of the stride velocity

Comparison on all available data including longitudinal from all participants. A: SMA = spinal muscular atrophy; B: CNM = centronuclear myopathy; C: FSHD = facioscapulohumeral muscular dystrophy; D: LGMD = Limb girdle muscular dystrophy; percSpeed = SV95V

Figure 43: Comparison of SV95C Between Weekday and Weekend



SV95C = 95th centile of the stride velocity

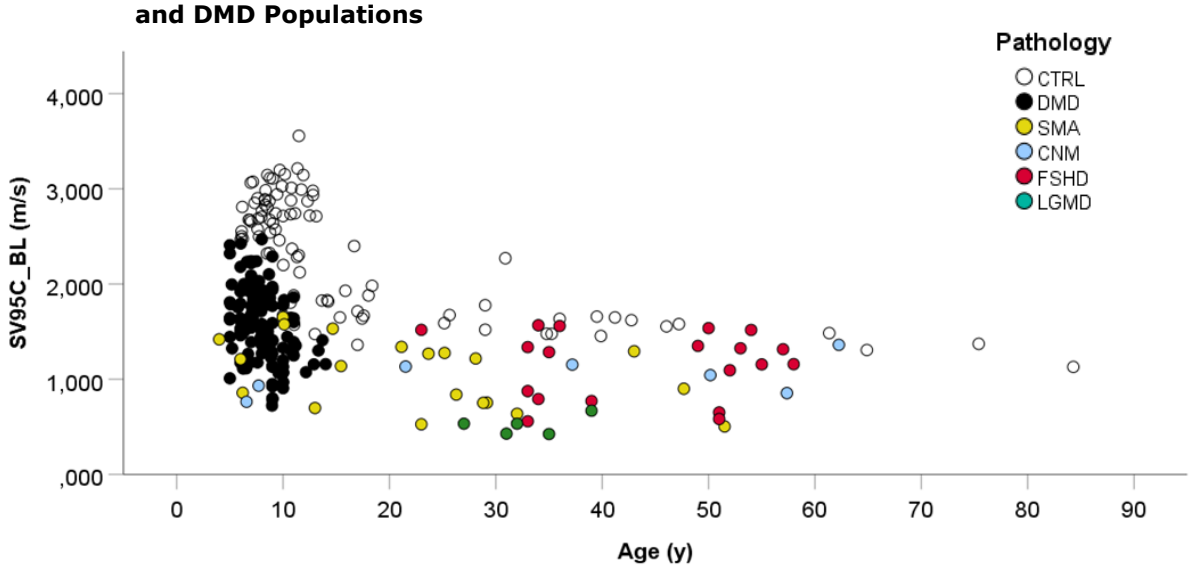
Comparison on all available data including longitudinal from all participants. A: SMA = spinal muscular atrophy; B: CNM = centronuclear myopathy; C: FSHD = facioscapulohumeral muscular dystrophy; D: LGMD = Limb girdle muscular dystrophy; percSpeed = SV95V

4.2.2.4. Construct Validity

4.2.2.4.1. Known-Groups Validity

Patients with SMA, CNM, FSHD, or LGMD were generally older than patients with DMD. In addition, the SV95C of patients with other NMDs was smaller than control subjects without any muscle condition and children with DMD (Figure 44).

Figure 44: Age Distribution vs SV96C in the Other NMD Populations and the Healthy Control and DMD Populations



CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy

To confirm the known-groups validity of the SV95C, pairwise comparisons of pathology were reported at Baseline between patients with other NMDs and healthy control subjects or DMD patients. Overall, as observed in DMD population, the results confirmed that SV95C, as well as other functioning outcome measures used in clinical trials (6MWD, and 4SC test), were able to discriminate patients with other NMDs from the healthy control population (Table 85, Table 86).

Specifically, based on pairwise comparisons, SV95C of patients with DMD or other NMD conditions was significantly smaller than SV95C of healthy subjects without any muscle condition (all P-values < 0.001). Other significant differences were also reported between the DMD population and the LGMD, SMA, and FSHD populations (P-values < 0.05; Table 86 and Figure 45).

Table 85: Comparison of SV95C, 6MWD, and 4SC Between DMD, the Other NMDs Populations, and Healthy Control Populations at Baseline (All Participants)

	N	Mean	Median	SD	Min	Max	p-value*
SV95C (m/s)							
DMD	125	1.571	1.563	0.382	0.700	2.500	**<0.001**
CTRL	93	2.338	2.500	0.606	1.129	3.556	
SMA	20	1.069	1.174	0.359	0.503	1.652	
CNM	7	1.034	1.043	0.203	0.763	1.361	
FSHD	19	1.155	1.284	0.348	0.558	1.567	
LGMD	5	0.517	0.533	0.100	0.424	0.669	
6MWD (m)[1]							
DMD	109	389.4	389.0	75.6	25.0	512.0	**<0.001**
CTRL	90	599.5	601.5	74.5	436.0	821.0	
CNM	7	318.0	377.0	133.7	150.0	463.0	
FSHD	13	446.8	486.0	102.2	178.0	530.0	
LGMD	-	-	-	-	-	-	
SMA	17	333.1	390.0	121.8	100.0	473.0	
4SC (m/s)[2]							
DMD	109	3.86	3.40	1.63	1.29	8.70	**<0.001**
CTRL	8	1.44	1.39	0.30	1.16	2.03	
SMA	15	10.17	6.03	10.60	1.85	36.09	

4SC = 4 stair climb test; 6MWD = 6-minute walking distance; CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SD = standard deviation; SV95C = 95th centile of the stride velocity

[1]6MWD was not available for LGMD population (6MWT not done by this population)

[2]4SC was only performed by SMA patients

Table 86: Comparison of SV95C Between DMD, the Other NMDs Populations, and Healthy Control Populations at Baseline (All Participants) - Post Hoc Analysis

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
LGMD-CNM	36.525	44.516	0.820	0.412	1.000
LGMD-SMA	-48.050	39.043	-1.231	0.218	1.000
LGMD-FSHD	58.189	39.248	1.483	0.138	1.000
LGMD-DMD	117.080	35.613	3.288	0.001	**0.015**
LGMD-CTRL	197.303	35.848	5.504	<0.001	**<0.001**
CNM-SMA	-11.525	32.666	-0.353	0.724	1.000
CNM-FSHD	-21.664	32.911	-0.658	0.510	1.000
CNM-DMD	-80.555	28.477	-2.829	0.005	0.070
CNM-CTRL	-160.778	28.771	-5.588	<0.001	**<0.001**
SMA-FSHD	10.139	25.016	0.405	0.685	1.000
SMA-DMD	69.030	18.806	3.671	<0.001	**0.004**
SMA-CTRL	149.253	19.247	7.755	<0.001	**<0.001**
FSHD-DMD	58.891	19.228	3.063	0.002	**0.033**
FSHD-CTRL	139.114	19.659	7.076	<0.001	**<0.001**
DMD-CTRL	80.223	10.693	7.502	<0.001	**<0.001**

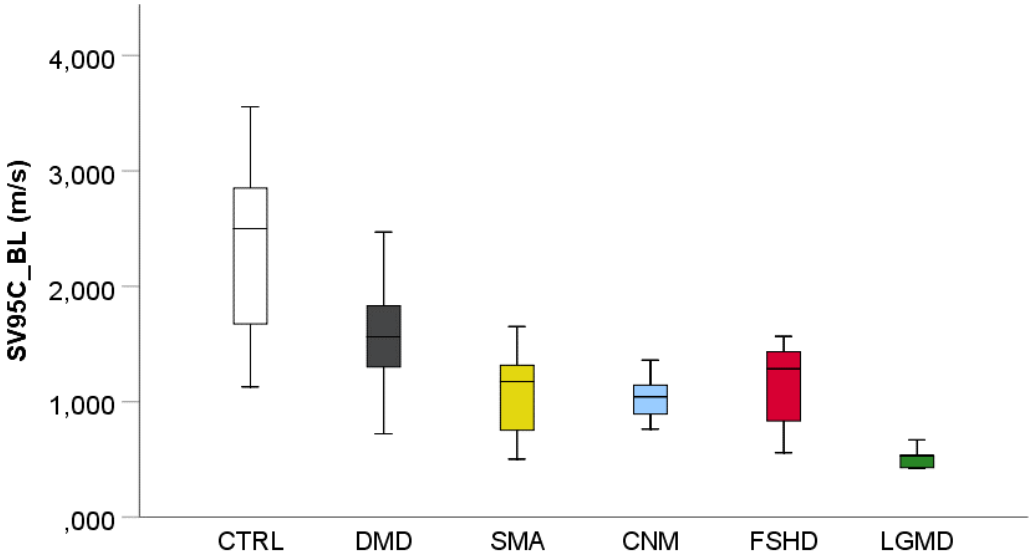
CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05.

[a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 45: Distribution of SV95C per NMDs Condition as Compared with Healthy Control Populations at Baseline



CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Similarly, as for DMD, when stratified by age groups (5 to 17 years of age [Table 87, Table 88, and Figure 46] and 18 to 84 years of age [Table 89, Table 90, Figure 47]), the SV95C of patients with other NMD conditions was significantly smaller than the SV95C of healthy subjects without any muscle condition (all P-values < 0.05).

Table 87: Comparison of SV95C Between the DMD, other NMDs, and Healthy Control Populations at Baseline (5 to 17 years)

	N	Mean	Median	SD	Min	Max	p-value*
DMD	125	1.571	1.563	0.382	0.70	2.50	**<0.001**
CTRL	73	2.539	2.676	0.512	1.361	3.56	
CNM	2	0.848	0.848	0.120	0.76	0.93	
SMA	8	1.260	1.315	0.348	0.70	1.65	

CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SD = standard deviation; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

NB: No patients with FSHD nor LGMD in this subgroup.

Table 88: Comparison of SV95C in CNM and SMA vs DMD and Healthy Control Populations at Baseline (5 to 17 Years) - Post Hoc Analysis

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
CNM-SMA	-38.375	47.583	-0.806	0.420	1.000
CNM-DMD	-70.712	42.899	-1.648	0.099	0.596
CNM-CTRL	**-155.370**	**43.139**	**-3.602**	**<0.001**	**0.002**
SMA-DMD	32.337	21.950	1.473	0.141	0.844
SMA-CTRL	**116.995**	**22.416**	**5.219**	**<0.001**	**<0.001**
DMD-CTRL	**84.658**	**8.866**	**9.549**	**<0.001**	**<0.001**

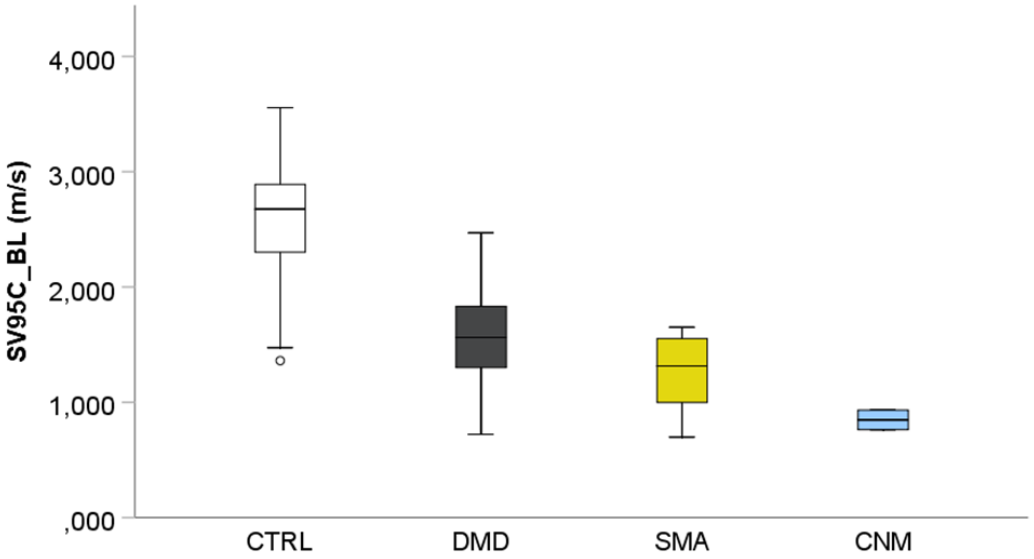
CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 46: Distribution of SV95C per NMDs condition as compared with Healthy Control Populations at Baseline (Age Range 5 to 17 Years)



CNM = centronuclear myopathy; CTRL = control; DMD = Duchenne muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Table 89: Comparison of SV95C Between the DMD, other NMDs, and Healthy Control Populations at Baseline (18 to 84 Years)

	N	Mean	Median	SD	Min	Max	p-value*
CTRL	20	1.604	1.584	0.246	1.129	2.271	**<0.001**
CNM	5	1.109	1.133	0.184	0.854	1.361	
SMA	10	0.942	0.869	0.319	0.503	1.340	
FSHD	19	1.155	1.284	0.348	0.558	1.567	
LGMD	5	0.517	0.533	0.100	0.424	0.669	

CNM = centronuclear myopathy; CTRL = control; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SD = standard deviation SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

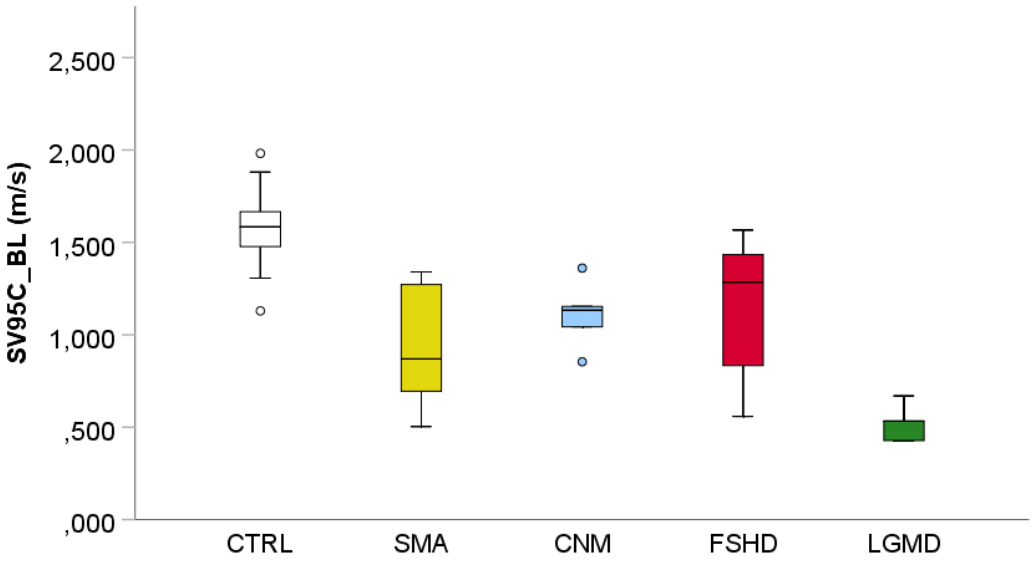
NB: No patients with FSHD nor LGMD in this subgroup.

Table 90: Comparison of SV95C Between the DMD, other NMDs, and Healthy Control Populations at Baseline (18 to 84 Years) - Post Hoc Analysis

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
LGMD-SMA	-13.917	9.450	-1.473	0.141	1.000
LGMD-CNM	19.400	11.228	1.728	0.084	0.840
LGMD-FSHD	23.842	8.923	2.672	0.008	0.075
LGMD-CTRL	**43.450**	**8.876**	**4.895**	**<0.001**	**<0.001**
SMA-CNM	5.483	9.450	.580	0.562	1.000
SMA-FSHD	9.925	6.546	1.516	0.129	1.000
SMA-CTRL	**29.533**	**6.482**	**4.556**	**<0.001**	**<0.001**
CNM-FSHD	-4.442	8.923	-.498	0.619	1.000
CNM-CTRL	**-24.050**	**8.876**	**-2.709**	**0.007**	**0.067**
FSHD-CTRL	**19.608**	**5.687**	**3.448**	**0.001**	**0.006**

CNM = centronuclear myopathy; CTRL = control; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

Figure 47: Distribution of SV95C per NMDs Condition as Compared with Healthy Control Populations at Baseline (Age Range 15 to 65 Years)



CNM = centronuclear myopathy; CTRL = control; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; NMD = neuromuscular disease; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

4.2.2.4.2. Convergent Validity

Table 91 presents correlation coefficients between the SV95C and the existing COAs (6MWD, 4SC, NSAA, MFM and Vignos) in a 1-month period around the onsite visit in SMA, CNM, FSHD, and LGMD patients. Based on available data, the SV95C measured in SMA, CNM, and FSHD was significantly correlated with the 6MWD, NSAA, 4SC, and MFM (P-values < 0.05). The relationship between SV95C and existing COAs appeared even stronger with SMA, CNM and FSHD populations as compared with the observations in the DMD population confirming the clinical relevance of SV95C also in other progressive NMDs with proximal muscle weakness. The good correlation with the more global function scale MFM that was used as a primary endpoint in pivotal trials in SMA and in clinical research in several diseases, suggests that the SV95C is relevant to describe the global functional ability of a patient with progressive NMDs characterized by a proximal muscle weakness. Despite a correlation coefficient of -0.7, no statistical significance was observed with the Vignos scale for the FSHD and LGMD populations but a significant strong correlation was previously published in the same population when LGMD and FSHD population were assessed together (R = 0.866; Appendix Section 7.6).[47] These results are also displayed graphically in Figure 48.

Table 91: Correlations Between SV95C and Other Functioning Outcome Measures (DMD and Other NMD Populations)

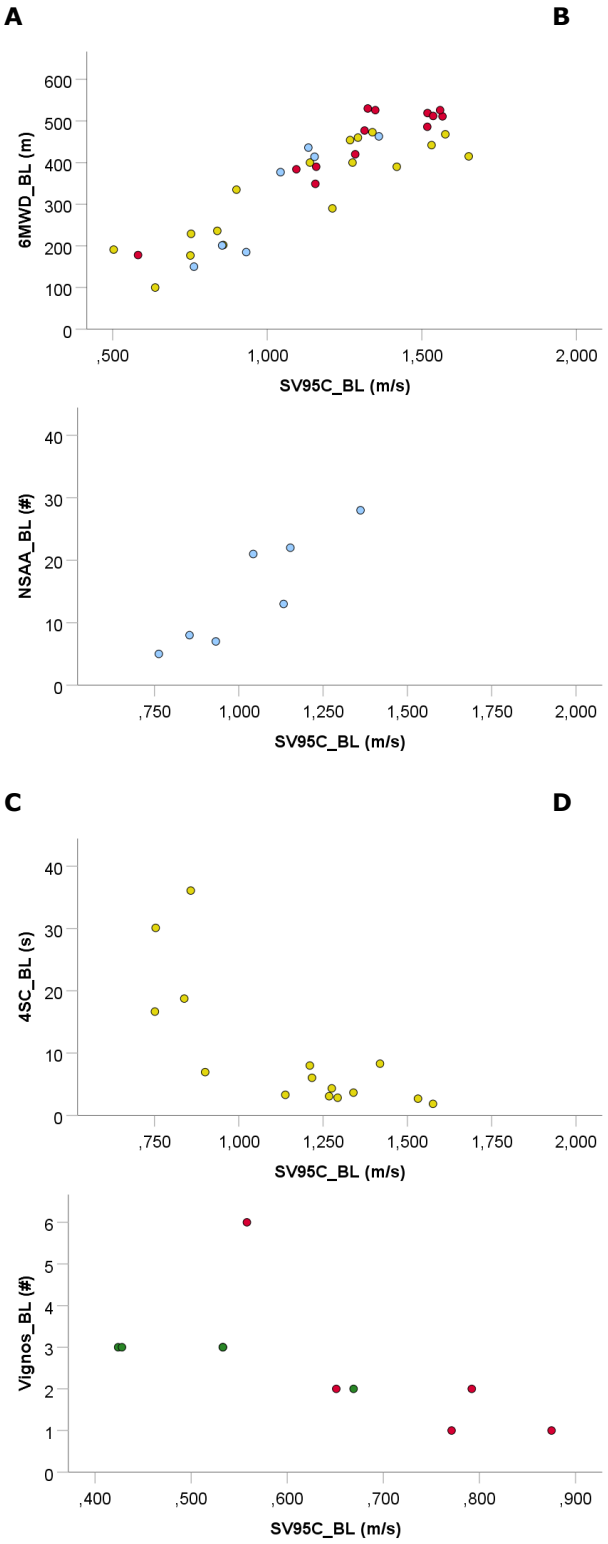
SV95C		6MWD	NSAA	4SC	MFM	Vignos
DMD *(refer Section 3.2.2.3.2)*	*Spearman's Rho*	*0.678***	*0.676***	*-0.622***	-	-
	Sig. (bilat)	*<0.001*	*<0.001*	*<0.001*	-	-
	N	*107*	*107*	*107*	-	-

SV95C		6MWD	NSAA	4SC	MFM	Vignos
SMA	Spearman's Rho	0.836**	-	-0.767**	0.790**	-
	Sig. (bilat)	<0.001	-	0.001	<0.001	-
	N	14	-	14	15	-
CNM	Spearman's Rho	0.929**	0.929**	-	0.857*	-
	Sig. (bilat)	0.003	0.003	-	0.014	-
	N	7	7	-	7	-
FSHD	Spearman's Rho	0.770**	-	-		-0.738
	Sig. (bilat)	0.002	-	-		0.155
	N	13	-	-		5
LGMD	Spearman's Rho	-	-	-		-0.725
	Sig. (bilat)	-	-	-		0.165
	N	-	-	-		5

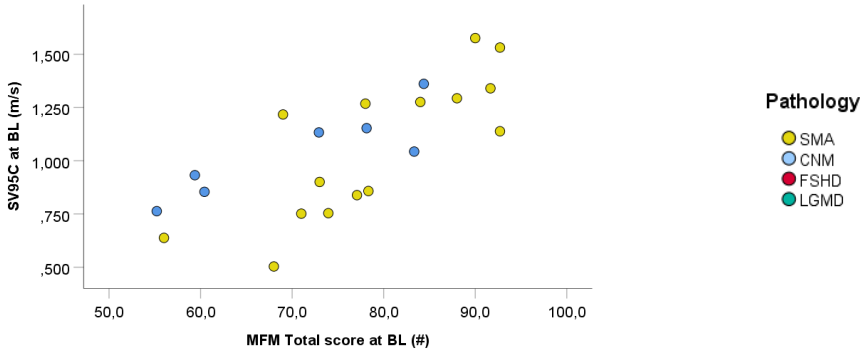
4SC = 4 stair climb test; 6MWD = 6-minute walking distance; CNM = centronuclear myopathy; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; MFM = Motor Function Measure; NMD = neuromuscular disease; NSAA = North Star Ambulatory Assessment; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

**Correlation is significant at the 0.01 level (2-tailed).* Correlation is significant at the 0.05 level (2-tailed)

Figure 48: Relationship Between SV95C and Other Functioning Outcome Measures



E



4SC = 4 stair climb test; 6MWD = 6-minute walking distance; BL = Baseline; CNM = centronuclear myopathy; DMD = Duchenne muscular dystrophy;
FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb girdle muscular dystrophy; MFM = Motor Function Measure; NMD = neuromuscular disease; NSAA = North Star Ambulatory Assessment; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

A: 6MWD; B: NSAA; C: 4SC; D: Vignos; E: MFM

4.2.2.5. Responsiveness (Ability to Detect Change)

4.2.2.5.1. Population

Flow charts of all available data for patients with SMA and CNM are provided in Figure 49 and Figure 50, respectively.

Figure 49: Available Data for SMA Populations

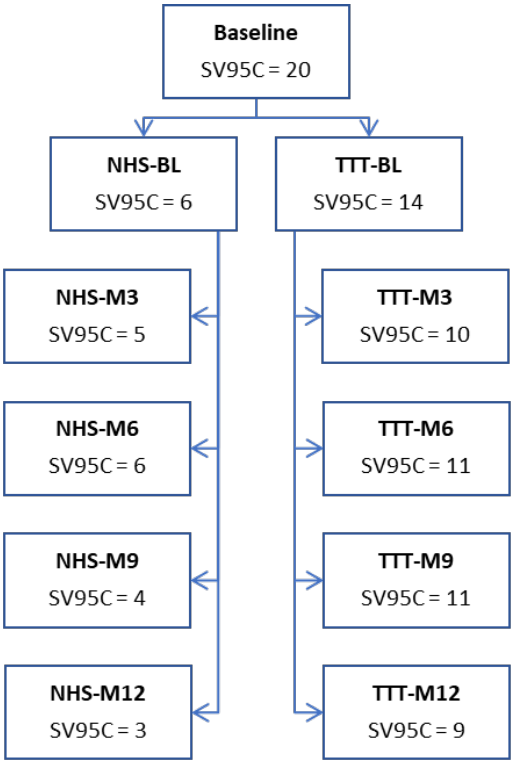
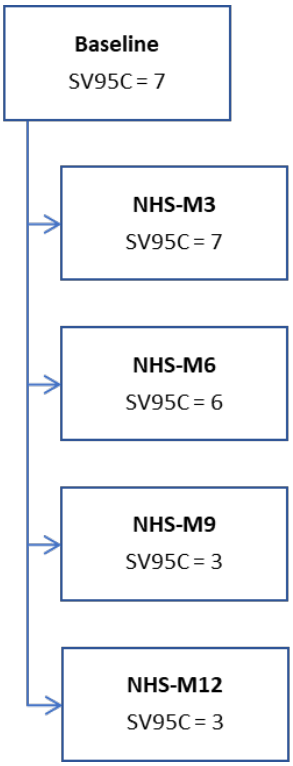


Figure 50: Available Data for CNM Population



4.2.2.5.2. Natural Change over Time

Responsiveness of the SV95C was determined by using the natural change over time at 3, 6, 9, and 12 months in 5, 6, 4, and 3 SMA patients (NHS-SMA-A study), and in 6, 6, 3, 3 CNM patients (NHS-CNM-A study) respectively. Overall, even if some caution need to be taken in drawing conclusions with such small sample sizes, SV95C tended to decrease continuously over time. Due to the slower disease progression, as expected, declines in SV95C were smaller in SMA and CNM as compared with DMD populations. The small changes and the reduced sample size contributed to the lack of any statistical significance (Table 92, Table 93, and Figure 51). Similarly, a global trend to decline in SV95C over 4 months with a statistically significant decline at 3 month of follow up, was previously published in the global FSHD and LGMD population from study CT-FSHD-A (Appendix Section 7.6).[47]

Table 92: SV95C Changes Over Time in Untreated SMA Patients

SV95C Change	3M	6M	9M	12M
N	5	6	4	3
Median	0.002	-0.021	-0.026	-0.046
Mean	-0.025	-0.050	-0.042	-0.008
SD	0.067	0.105	0.092	0.109
p-value*	0.600	0.463	0.465	1.000

SD = standard deviation; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

**One-sample Wilcoxon signed rank test*

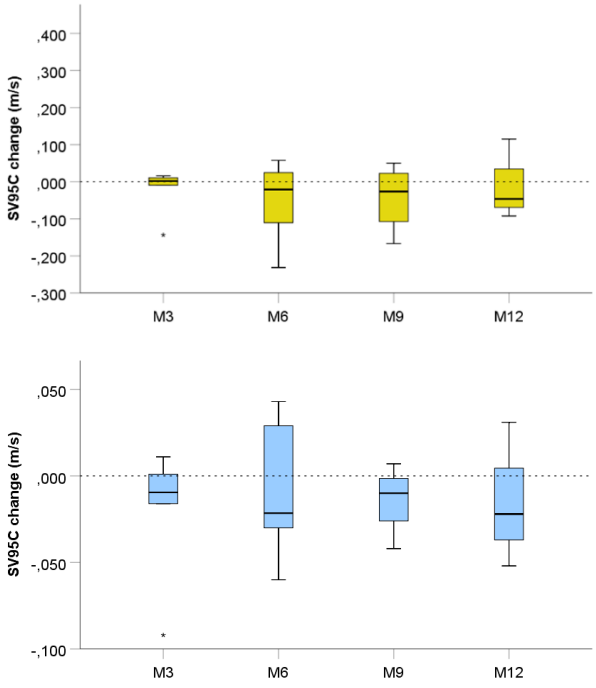
Table 93: SV95C Changes over Time in Untreated CNM Patients

SV95C Change	3M	6M	9M	12M
N	6	6	3	3
Median	-0.010	-0.022	-0.010	-0.022
Mean	-0.019	-0.010	-0.015	-0.014
SD	0.0370	0.0387	0.025	0.042
p-value*	0.173	0.600	0.285	0.593

CNM = centronuclear myopathy; SD = standard deviation; SV95C = 95th centile of the stride velocity

**One-sample Wilcoxon signed rank test*

Figure 51: SV95C Change Over Time in Patients with SMA or CNM in the Natural Course of the Disease



CNM = centronuclear myopathy; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

4.2.2.5.3. Positive Change

The sensitivity of SV95C to a positive change was assessed in 10 patients with SMA who were treated with Spinraza® (NHS-SMA-B study). In this small sample, the SV95C remained stable over time, but it is difficult to draw meaningful conclusions based on the

small sample size (Table 94, Figure 52).

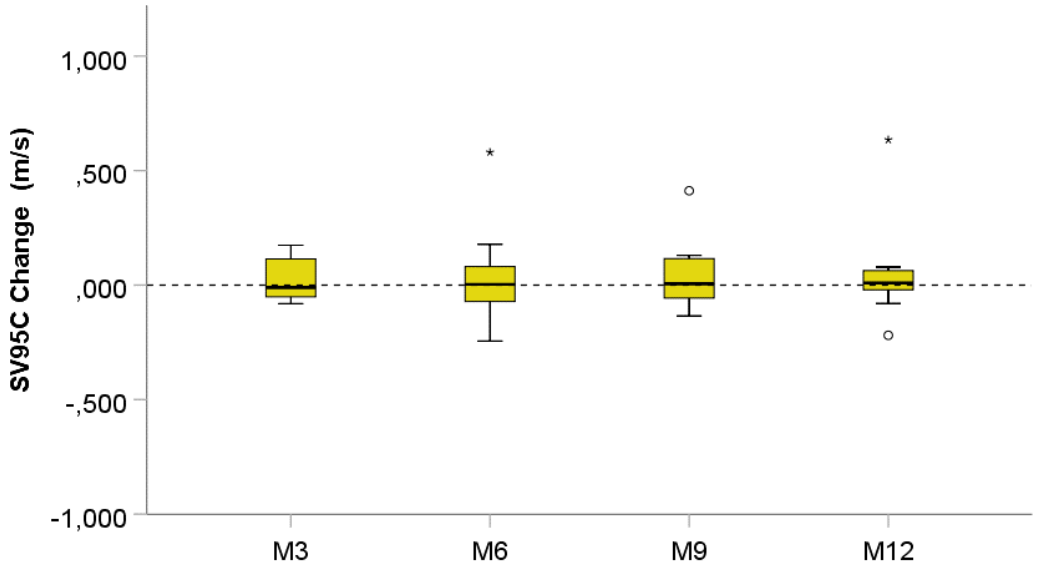
Table 94: SV95C Changes Over Time in SMA Patients Treated with Spinraza®

SV95C Change	3M	6M	9M	12M
N	10	11	10	9
Median	-0.010	0.004	0.006	0.009
Mean	0.020	0.039	0.044	0.056
SD	0.087	0.215	0.159	0.235
p-value*	0.721	0.790	0.575	0.767

SD = standard deviation; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

**One-sample Wilcoxon signed rank test*

Figure 52: SV95C Change Over Time in Patients with SMA Treated by Spinraza®



SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

4.2.2.6. Distribution-based Meaningful Change Thresholds

Overall, the SEM was smaller in the SMA, CNM, FSHD, and LGMD populations than in patients with DMD (0.032 m/s, 0.060 m/s, 0.022 m/s, and 0.032 m/s, respectively versus 0.070 m/s), although the SEM in the CNM population at 0.060 was more like that in DMD. This overall difference might be due to the severity of the disease but also to the age of the population. Indeed, those populations are mostly composed of adult patients and it was demonstrated that SV95C is smaller in adult population probably due to limited episodes of running in adults.[40] Consequently, the MDCs at 80%, 90%, and 95% of level of confidence were lower in SMA, CNM, FSHD and LGMD than in DMD populations, particularly in SMA, FSHD, and LGMD, and similar to each other (ranging from 0.040 to 0.089); the MDCs for

the CNM population (0.110 to 0.167) were more similar to those in DMD (0.127 to 0.194; Table 95).

Table 95: SV95C SEM and MDC in the DMD and Other NMD Populations

	DMD ***(refer Section 3.2.2.5.1)***	**SMA**	**CNM**	**FSHD**	**LGMD**
N	*103*	19	6	33	4
ICC*	*0.962*	0.990	0.941	0.992	0.967
95% CI	*[0.943–0.974]*	[0.975-0.996]	[0.585–0.992]	[0.983-0.996]	[0.674-0.998]
SV95C-Period1 mean (m/s)	*1.467*	1.023	1.006	1.314	1.062
SV95C-Period1 SD (m/s)	*0.360*	0.322	0.249	0.248	0.177
SEM (m/s)**	***0.070***	**0.032**	**0.060**	**0.022**	**0.032**
SEM relative to RP1 (%)	*4.78*	3.15	6.00	1.69	3.02
MDC80 % (m/s)	***0.127***	**0.059**	**0.110**	**0.040**	**0.058**
MDC90 % (m/s)	*0.163*	0.075	0.141	0.051	0.075
MDC95 % (m/s)	*0.194*	0.089	0.167	0.061	0.089

CI = confidence interval; CNM = centronuclear myopathy; DMD = Duchenne muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; ICC = intra-class correlation; LGMD = limb girdle muscular dystrophy; MDC = minimal detectable change; NMD = neuromuscular disease; RP = recording period; SD = standard deviation; SEM = standard error of measurement; SMA = spinal muscular atrophy; SV95C = 95th centile of the stride velocity

* Intra-class correlation coefficient – 2-way random effect model, absolute agreement, average measure

** SEM = SD*SQR(1-ICC)

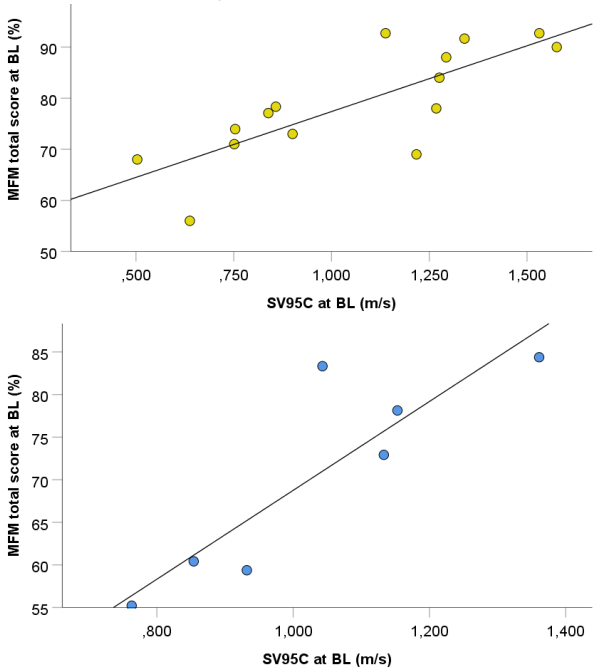
*** MDC=z-score*SEM*SQR(2) with z-score = 1.960, 1.645, and 1.282 at 95%, 90%, 80% confidence levels, respectively.

Consequently, a MCT around 0.1 m/s seems also acceptable for other progressive NMDs with proximal muscle weakness. This finding is supported with our results suggesting that a SV95C change of 0.1m/s corresponds to changes of MFM total score of 2.6 and 5.2 points for SMA and CNM respectively, when a change of 3 points in MFM was previously demonstrated as clinically significant in SMA[49] (Figure 53).

Figure 3: Linear Regressions between SV95C and MFM total score

A **B**

y = 51.63 + 25.75*x ; R² = y = 16..6 + 52.16^{E2} *x ; R²



BL = Baseline; MFM = MotorFfunction Measure, SV95C = 95th centile of the stride velocity

A: SMA patients; B: CNM patients

5. Remaining Gaps and a Brief Overview of how these will be Addressed

This qualification is based on data collected on patients older than 5 years and additional data is required to extend the validity of SV95C to younger ambulant patients with DMD. Indeed, with the walking ability acquisition, growth, and the relatively low impact of the disease in children aged 2 to 5, the evolution of SV95C is not yet established and might present more likely with an improvement and a higher variability.

The MCT of SV95C in DMD was established based on a distribution and anchor-based methods supported by the changes observed during the natural course of the disease and after starting the corticosteroids. Unfortunately, patient and clinician reported outcome measures used in this analysis were collected during a clinical trial which was prematurely stopped; this may introduce a bias in the perception of global clinal state of patients leading to an overestimated MCT of 0.1 to 0.3 m/s. Nevertheless, a decline of 0.110 m/s and 0.204 m/s was reached after 9 and 12 months of follow up in untreated weakening DMD patients and the improvement of SV95C in DMD patients having starting corticosteroids was of 0.090 m/s at 3 months, 0.211 m/s at 6 months, and 0.307 m/s at 12 months of follow up indicating that the proposed MCT is reachable in the current timeframe of clinical trials.

Collecting additional data with patient reported outcome through health-related quality of life questionnaires will help to strengthen the anchoring and refinement of a MCT for the SV95C.

DMD is a progressive condition in which loss of ambulation is an important milestone. Several studies have demonstrated that the age at loss of ambulation is predictive of the age at which future significant milestones are met such as the need of assisted ventilation and life expectancy. We are currently gathering data to try to understand how SV95C can be predictive of age at loss of ambulation. The qualification of SV95C as a secondary endpoint has helped to include this measure in several clinical trials. Qualification as a primary endpoint with further reinforce this trend and will be decisive in acquiring the amount of data that are needed to assess this important question. We also suggest, as recommended on the EMA guidance for clinical investigations in Duchenne and Becker muscular dystrophy8 published by EMA, to use a relevant secondary endpoint assessing muscle or strength function in the design of the future clinical trials using SV95C as a primary endpoint to confirm consistency.

Findings observed with the DMD population were confirmed by results on the other progressive NMDs characterized with proximal muscle weakness as SMA, CNM, FSHD and LGMD indicating that SV95C is also a relevant outcome measure for those population. Nevertheless, additional data including a broader range of disabilities and patients are needed to confirm the first conclusions and the level of minimal clinical relevance.

6. Conclusions

Based on the entire set of evidence presented here and previously in the original application, SV95C is an accurate digital and clinically meaningful outcome assessment for use as a primary endpoint in clinical trials targeting ambulant patients with DMD and should be qualified as a valid secondary endpoint in patients with other NMDs involving progressive proximal muscle weakness of lower limb such as SMA, CNM, LGMD and FSHD. Evidence is presented to show that ambulation is a key aspect of DMD (and other progressive NMDs) and that all key stakeholders (patients, caregivers, clinicians, patient advocacy groups, industry and regulators) agree that there is a need for a validated COA measure to assess mobility in this population that more accurately reflects the patients daily functioning in real-life, that is not limited to performance on a clinic-based assessment completed at a specific point in time in an artificial setting, and that reduces the burden on sites, staff and patients completing these tests.

The SV95C is a measure that addresses these limitations with existing COA measures that are used. Additional evidence has been presented, building upon data that was presented and reviewed as part of the previous secondary endpoint qualification opinion package (EMA/CHMP/SAWP/178058/2019), in which it was demonstrated that the SV95C when measured with the wearable ActiMyo® device is accurate, reliable, sensitive to change, and clinically relevant based on the correlations to existing COAs of established clinical relevance. This additional evidence has been presented to address the comments raised by the CHMP at that time, to confirm and further inform the different measurement properties of the SV95C, and to support its use as a primary endpoint to assess new drug efficacy in clinical trials from Phase 1 to Phase 4 for the measurement of the maximal stride velocity of patients with DMD.

Qualitative data from patients and caregivers and feedback from HCPs have confirmed the relevance of ambulation, walking speed and content validity of SV95C in the DMD population. Feedback has shown that patients and caregivers recognize and value the use of a real-life based wearable device such as ActiMyo® and would be willing to use such in a clinical trial setting. In addition, patient and caregiver

[8] Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy, 2015

data has been presented showing that this is consistent with other progressive NMDs in which ambulation is affected. Quantitative data from studies involving greater numbers of DMD patients and lengthier follow up has been presented, which confirm the psychometric properties of the SV95C previously presented in the secondary endpoint qualification package. Furthermore, this evidence extends to other NMDs, illustrating the generalizability of these findings across conditions.

Based on the totality of evidence presented, we demonstrate that SV95C is an accurate digital and clinically meaningful outcome tool assessing passively the maximal speed of a patient in a real-life setting through a medical device worn by ambulant patients living with DMD. The evidence supports its use as a primary efficacy endpoint in clinical trials targeting ambulant patients with DMD. Similar data obtained in other rare NMDs involving proximal progressive weakness shows SV95C relevance and validity, which should prompt secondary endpoint qualification until more data are provided to support full primary qualification.

CHMP discussion

a. General comments

The advantages of the SV95C as indicator of ambulatory function in contrast to its alternatives, i.e. six-minute walking test (6MWT), North Star Ambulation Assessment (NSAA) or 4 stairs climbing (4SC), are acknowledged. Especially, the SV95C allows continuous monitoring over relatively long period in a home-setting and therefore is less sensitive to the moment of the day and relies less on patient motivation or subjective assessment.

For acceptance as primary efficacy endpoint at the time of Qualification as secondary endpoint CHMP requested further data on

- long-term performance and correlation with functional tests,
- normative data, and
- the sensitivity to change and the clinical relevance of the postulated minimal clinical important difference (MCID) of 0.1 m/s.

Moreover, since the sensors of the system could record additional data apart from stride velocity the Applicant was also encouraged to generate data on quality of walking, fall, sway, real world stairs, time to stand and correlation with patient well-being. The Applicant was also encouraged to conduct further work in younger and non-ambulant patients. Data on these two aspects have not been provided in this latest qualification submission. The Applicant states that no limitation is foreseen for younger patients if they accept wearing the device long enough to collect enough data. However, a feasibility report was not provided and considering the potential impact of developmental issues such as walking ability acquisition and growth, the performance characteristics in a population older than 5 years of age cannot be transferred to younger patients (e.g. 2-5 years of age).

b. Qualitative research

Dedicated online surveys were undertaken with patients or caregivers (n=549) and health care providers (HCPs, n=52) to establish content validity of the SV95C. These surveys also included other NMDs. In addition, several supporting letters from experts (n=8) were provided.

With respect to SV95C as a measure of ambulation, content validity and face validity are not straightforward, : ambulation has many features, and it is difficult to imagine to which extent a change in SV95C translates in delay in progression or improvement of these features e.g. walking difficulties, falls, endurance, muscle strength, ADL activities. In fact, ambulation and maximal speed were considered by all groups as clinically meaningful outcomes. Change in stair-climb, limiting falls, the

ability to self-transfer and walking ability in itself were considered more important than stride velocity. For patients with DMD fatigue during ambulation and distance walked before stopping plus distance walked during the day were considered to best represent an improvement. It appears that for the justification of the content validity of the SV95C the relevant questions to which extent stride velocity may be translated in an improvement in for instance walking quality were not posed, e.g. a question on impact on action radius was not asked.

Nevertheless, the face and content validity of the SV95C is not at discussion considering that the SV95C has already been qualified as secondary endpoint in ambulatory DMD studies and it is clear that the SV95C is highly correlated to the 6MWT, the up to now most commonly used primary endpoint in studies in ambulatory DMD.

Thus, overall results are supportive for use of a wearable device to assess walking related abilities. This would also include other ambulation related endpoints, e.g. total walking distance, distance covered with walking bouts, stair climbing.

c. Quantitative evidence

Data from the 45 European DMD patients as assessed in the previous qualification request are supplemented with data from 80 additional patients from US, EU and Australia (n=125 patients overall) either stable or starting on corticosteroids. Data from 66 healthy age-matched controls are also presented. The data originate from 7 clinical trials including Natural History Data and data from in-clinic patients (Appendix 7.8).

In the initial submission only data for patients of 5 years and older were provided. This was the reason to limit the context of use to children ≥5 years of age. During the consultation phase new data were submitted, indicating that compliance is not different for DMD subjects between 4 and 5 years of age (n=29) as compared to DMD subjects between 5 and7 years of age (n=100). It is not expected that for children between 4 and5 years of age, who are able to wear the device appropriately, the performance of the SV95C is different. Hence, there is no objection to the lower age limit to 4 years of age.

The quantitative analyses on accuracy, test-retest reliability, robustness and known-groups validity had also been presented during the first procedure and found acceptable to qualify SV95C as a secondary endpoint. They have now been supplemented with further data claimed to confirm the findings and sufficient for SV95C qualification as a primary efficacy endpoint. The ability to detect change during the natural course of the disease had already been shown in the previous procedure and has now been confirmed in a larger dataset with longer follow up. Moreover, the ability to detect change due to treatment improving the condition was evaluated in 11 patients who started treatment with corticosteroids. While the number of patients was small, a statistically significant increase from baseline was seen at months 3 and 6. Although these results have been derived in an uncontrolled setting, they document improvement rather than the deterioration as seen in the natural course of disease investigations.

Convergent validity was assessed in data from 3 natural history studies and 2 clinical trials without observed efficacy of the test treatment (n=107).

Comparisons between SV95C and established COA show reasonable correlations with comparable correlation coefficients in the range of 0.63 to 0.68 (ρ). Correlation plots show the same shape and suggest lower correlation to e.g. 6MWT with higher SV95C values. This is not unexpected as different aspects of walking performance are addressed.

Assessment of longitudinal data after baseline at 3, 6 and 12 months show a larger range of correlation coefficients for COAs as expected with smaller data sets at later time points.

Responsiveness to change was assessed in a natural history cohort with a considerable number of patients from 3 natural history studies and 2 clinical trials without observed efficacy of the test treatment (N=81 at 3 months to N=28 at 12 months), allowing comparisons to 6MWT and NSAA in a subset of these patients up to 12 months after baseline assessment (minimum number of patients in the data set were N=43 at 3 months and N=15 at 12 months). All these patients were on a stable corticosteroid therapy.

Of note, it is of some concern that the correlation between change in SV95C and change in 6MWT, NSAA and 4SC over time is rather poor. The Applicant explains that this was not unexpected as only SV95C shows significant decreases in shorter time periods. It is argued that 6MWT, NSAA and 4SC are less sensitive to change. This argument is partly agreed as, based on the responsiveness data presented, the 6MWT, NSAA and 4SC are sensitive to change over a follow-up of 12 months (see figure 17, 19 and 21). At least with respect to change in SV95C and change in 6MWT, NSAA and 4SC at 12 months a better correlation than the one observed would have been expected if not already at the 9-month timepoint. Longer follow-up data may address this further.

The responsiveness of SV95C to a positive change was assessed in a limited cohort of 11 patients with DMD who started corticosteroid therapy. A significant positive change in SV95C at 3 months and at 6 months from baseline was observed based on the median SV95C change scores. Changes at 9 and 12 months were non-significant. Although limited by small sample size, these results suggest some sensitivity of SV95C to detect positive changes of an intervention. While the sample size is small and limits robustness of conclusions, results overall suggest usefulness.

Minimal detectable change was assessed with distribution-based methods in a large data set of N=103 patients from 3 natural history studies, 3 clinical trials and additional patients followed in an in-clinic setting. Minimal change threshold (MCT) was assessed with an anchor-based method using CGI-C and PODCI subdomain ('transfers and basic mobility') data from a clinical trial with a treatment showing no efficacy with N=12 and N=15 patients, respectively.

Based on the results of the distribution- and anchor-based analyses it is suggested by the Applicant that a change in SV95C of at least ≈-0.10 m/s would be required for the change in DMD patients to be beyond measurement error evaluated at 0.07 m/s, and that a change score of between -0.10 and -0.20 m/s would be clinically meaningful.

The distribution-based threshold for meaningful change results in values roughly ranging from 0.1 to 0.2 m/s, depending on the age-group. Although an anchor-based threshold is preferred, this does indicate what threshold would be beyond measurement error.

The approach to derive an anchor based MCT clearly suffers from the fact that the clinical trial data come from a study prematurely stopped due to absence of efficacy. Further, the anchor-based approach to determine a within patient threshold is hampered by the low number of patients, specifically considering that the possible number of response categories were collapsed (i.e. for CGI-C: improved (n=4) /stable (n=5) /worsened (n=3); for PODCI improved (n=0) /stable (n=9) / worsening (n=6).

A correlation was observed for the absolute values of SV95C at week 48 and both CGI-C and PODCI. Like the convergent validity, no correlations with change from baseline were observed, again suggesting that the SV95C endpoint could be used best applying the absolute value instead of change from baseline.

For the CGI-C the absolute values of SC95% at week 48 discriminated between subjects scoring minimally improved, no change and being worse. However, the change in SV95C at 48 weeks was not able to separate between improvement, no change, and worsening of the CGI-C. The mean change in

SV95C at 48 weeks was -0.175 m/s, -3.02 m/s and -0.370 m/s for subjects in improved (n=4), stable (n=5) and worsening (n=3) CGI category. Moreover, the CGI-C categories were condensed. The mean difference in change in SV95C between stable subjects and subject who worsened (all scored much worse) was only 0.07 m/s. Thus, based on these data the proposal that a change score of between -0.10 and -0.20 m/s is a clinical meaningful threshold is not considered justified.

For the PODCI the same arguments as for the CGI-C apply, as the mean difference in change in SV95C between subjects stable or worsening in the PODCU was 0.072 m/s.

For the NSAA anchor the same arguments apply, since the mean difference of change in SV95C over week 48 between subjects stable and worsening on the NSAA was 0.018 m/s.

For the 6MWT 'anchor' the mean difference of change in SV95C between stable subjects and subjects who worsened (> 30 m) on the 6MWT was 0.147 m/s. However, the observation of a close relationship may be obvious considering high correlation between the SV95C and 6MWD and the SV95C may replace the 6MWT. An anchor-based method to establish the clinically meaningful change should not be based on two closely related measures on the same scale that are expressed largely with the same physical dimensions, i.e. distance walked over time has basically the same physical unit as speed.

It is noted that the analysis with 6MWT and NSAA as anchors exhibit variability and inconsistency if the same change between stable and worsened would be used as for the CGI-C and PODCI data (table 69).

Considering all data presented, there is only limited support from anchor-based methods for a properly derived MCT. That a change in SV95C between -0.10 and -0.20 m/s would be meaningful may be challenged.

d. Overall discussion

As stated, the advantages of the SV95C as indicator of ambulatory function are clear, i.e. the SV95C allows a continuous monitoring over a relatively long period in a home-setting and is therefore less sensitive to timing of the assessment (e.g. day and time of test) and relies less on patient motivation or subjective assessment when compared to established tests.

However, the content and face validity the SV95C is less clear. In fact, change in stair-climbing, ability to self-transfer and walking ability and fatigue appear more important to the patients/caregivers than maximal stride velocity. In addition, the notion that a change of -0.10 to -0.20 m/s would be clinically meaningful is challenged. Furthermore, the correlation of change in SV95C and change in 6MWT, NSAA and 4SC is not clearly established by data submitted for qualification, probably due to the limited duration of the longitudinal follow-up in a limited number of subjects.

Nevertheless, the SV95C is highly correlated to the 6MWT and is more sensitive as compared to the 6MWT, the up to now the most commonly used primary endpoint in studies in ambulatory DMD. As such, the SV95C may be considered an alternative endpoint to the 6MWT in studies in DMD. Thus, the potential interchangeability between the SV95C and 6MWT would be the argument in favour of the SV95C as an alternative primary endpoint in DMD studies. Thus, qualification of the SV95C as primary endpoint in studies in ambulatory DMD can be considered for this reason.

However, it is questioned whether the SV95C is acceptable as the sole decisive endpoint in efficacy studies in ambulatory DMD. From a methodological perspective the primary endpoint is the variable for which the study is powered and if statistical significance is met, the study would be considered successful. From a clinical perspective the primary endpoint is the endpoint that reflects /represents the underlying condition best, and if an effect on the primary endpoint is observed, then it would be concluded that an effect on the underlying condition is clear. In DMD, these two perspectives do not

fully coincide. For this reason, it is expected also for the 6MWT, the traditional primary endpoint in ambulatory DMD, that an effect on the primary endpoint should be confirmed/ supported by results from secondary endpoints. Hence, efficacy still will be concluded based on the totality of the evidence collected and presented.

Of note, the Applicant indicated during the discussion meeting that further research is intended to further substantiate the MCT and to evaluate the predictive value of the SV95C for functional milestones.

Acceptance of the SV95C variable is device agnostic provided accuracy and reliability of measurement are established.

In conclusion, considering all the above, a qualification of the SV95C as primary endpoint in superiority studies in ambulatory DMD as alternative to the 6MWT is considered acceptable provided that the usual connotation that if the primary endpoint is met the study is a success, is not made. As indicated in EMA Guideline EMA/CHMP/236981/2011, Corr. 11, "effects on the single selected primary endpoint should be supported by results from the most relevant secondary endpoints for consistency."

Qualification Opinion

CHMP qualifies the SV95C as primary endpoint in superiority studies in ambulatory Duchenne Muscular Dystrophy (DMD) as alternative to the 6 Minute Walk Distance (6MWD) provided this outcome measure is supported by consistent findings in established efficacy endpoints included as secondary endpoints.

For the full qualification opinion statement see section 1. above.