APPENDIX

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Study Group Information

Steering Committee

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- Prof. Fred LUBLIN, New York, USA
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- Prof. Jerry S. WOLINSKY, Houston, USA
- Prof. Mark S. FREEDMAN, Ottawa, Canada
- Prof. Giancarlo COMI, Milan, Italy
- Prof. Gavin GIOVANNONI, London, UK
- Prof. Hans-Peter HARTUNG, Düsseldorf, Germany

Data and Safety Monitoring Board (DSMB)

- Prof. Stephen REINGOLD, Salisbury, USA
- Prof. Pierre DUQUETTE, Montreal, Canada
- Prof. Tobias DERFUSS, Basel, Switzerland
- Prof. Franz FAZEKAS, Graz, Austria
- Prof. Maria Pia SORMANI, Genoa, Italy

Adjudication Committee

- Prof. Robert P. LISAK, Detroit, USA
- Dr. Jennifer GRAVES, San Diego, USA
- Dr. Stephen KRIEGER, New York, USA
- Dr. Rana K. ZABAD, Omaha, USA
- Dr. Scott NEWSOME, Baltimore, USA

Principle Investigators

- **Australia:** Joshua Barton, Richard MacDonell, Mark Marriott.
- **Belgium:** Nina De Klippel, Guy Laureys, Barbara Willekens.
- **Canada:** Virginia Devonshire, Mark Freedman, J Marc Girard, Paul Giacomini, Roger McKelvey, Daniel Selchen, Galina Vorobeychik, Ludivine Witkowski.
- **Czech Republic:** Radek Ampapa, Jana Lizrova Preiningerova, Eva Meluzinova, Radomir Talab, Marta Vachova.
- **Germany:** Orhan Aktas, Mathias Buttmann, Elias-Hamp Birte, Tania Kuempfel, Paul Friedemann, Daniela Rau, Gerd Reifschneider, Piotr Sokolowski, Hayrettin Tumani.
- **Hungary:** Maria Satori.
- **Italy:** Carlo Pozzilli.
- **Poland:** Agata Klosek, Jozef Koscielniak, Fryze Waldemar, Malgorzata Zajda.
- **Spain:** Rafael Arroyo Gonzalez, Guillermo Izquierdo Ayuso, Victoria Fernandez Sanchez, Celia Oreja Guevara, Jose Enrique Martinez Rodriguez, Xavier Montalban, Lluis Ramio-Torrenta.

- **Sweden:** Lou Brundin, Jan Lycke.
- **Turkey:** Murat Terzi.
- **United Kingdom:** Joe Guadagno, Don Mahad, Adrian Pace, Klaus Schmierer, Ahmed Toosy, Stewart Webb.
- **United States of America:** Mark Agius, Lilyana Amezcua, Michelle Apperson, Bridget Bagert, Daniel Bandari, Evanthia Bernitsas,Jonathan Calkwood, Jonathan Carter, Bruce Cohen, Devon Conway, Joanna Cooper, John Corboy, Patricia Coyle, Bruce Cree, Mitchel Freedman, Corey Ford, Edward Fox, Myla Goldman, Benjamin Greenberg, Mariko Kita, Thomas Leist, Sharon Lynch, Aaron Miller, Harold Moses, Robert Naismith, Mary Ann Picone, Bhatia Perminder, Alexander Rae-Grant, Kottil Rammohan, Anthony Reder, Claire Riley, Derrick Robertson, Vernon Rowe, Shiv Saidha, Lawrence Samkoff, Christopher Severson, Kyle Smoot, Sharon Stoll, Randall Trudell, Bianca Weinstock-Guttman, Sanjay Yathiraj.

Analysis centers

- Monitoring, data management, statistical analysis: Parexel International (Dublin, Ireland)
- Cardiac Safety Central Laboratory: Banook (Nancy, France)
- Core Imaging Laboratory: NeuroRx Research (Montreal, Canada)
- Clinical Central Laboratory: Eurofins (Lancaster, USA/Breda, The Netherlands/Singapore, Asia)
- Independent Biotin Assay Laboratory: Atlanbio (Saint Nazaire, France)
- The NIH-funded Eureka Research Platform (U2CEB021881) enabled step count collection and STEPS CORE interpreted the data (San Francisco, CA, USA).

Table S1: Full inclusion/exclusion criteria (IC/EC)

Inclusion
1. Patient aged 18-65 years old
2. Signed and dated written informed consent form in accordance with local regulations: having freely given their written informed consent to participate in the study
3. Diagnosis of primary or secondary progressive MS fulfilling revised McDonald criteria (2010) and Lublin criteria (2014)
4. Documented evidence of clinical disability progression within the 2 years prior to inclusion, i.e. a) progression of EDSS during the past two years of at least 1 point sustained for at least 6 months if inclusion EDSS is from 3·5 to 5·5 or at least 0·5 point increase sustained for at least 6 months if inclusion EDSS is from 6 to 6·5 or b) increase of TW25 by at least 20% in the past two years sustained for at least 6 months or c) other well-documented objective worsening validated by the Adjudication Committee
5. EDSS score 3·5 to 6·5 at inclusion
6. TW25<40 seconds at inclusion visit
7. Kurtzke pyramidal functional subscore ≥2 defined as “minimal disability: patient complains of motor-fatigability or reduced performance in strenuous motor tasks” (motor performance grade 1) and/or BMRC grade 4 in one or two muscle groups”
Exclusion
1. Clinical evidence of a relapse in 24 months prior to inclusion
2. Treatment with any product containing biotin as single ingredient within six months prior to inclusion (multivitamin supplementation authorized if biotin < 1mg per day)
3. Concomitant treatment with fampridine at inclusion or in the 30 days prior to inclusion
4. New immunosuppressive/immunomodulatory drug initiated less than 90 days prior to inclusion
5. Treatment with botulinum toxin (except for cosmetic purposes) initiated within 6 months prior to inclusion
6. In-patient rehabilitation program within the 3 months prior to inclusion
7. Pregnancy, breastfeeding or women with childbearing potential without acceptable form of contraception
8. Men unwilling to use an acceptable contraceptive method
9. Any general chronic handicapping/incapacitating disease other than MS
10.Any serious disease necessitating biological follow up with biological tests using biotinylated antibodies or substrates
11. Past history of rhabdomyolysis/metabolic myopathy
12. Known fatty acids beta oxidation defect
13. Known hypersensitivity or intolerance to biotin, analogues or excipients, patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption
14. Patients with hypersensitivity or contra-indication to gadolinium
15. Patients with uncontrolled hepatic disorder, renal or cardiovascular disease, or cancer
16. Laboratory tests out of normal ranges considered by the investigator as clinically significant with regards to the study continuation
17. Patients with history or presence of alcohol abuse or drug addiction
18. Untreated or uncontrolled psychiatric disorders, especially suicidal risk assessed by Columbia-Suicide Severity Rating Scale (C-SSRS)
19. Participation in another research study involving an investigational product (IP) in the 90 days preceding inclusion, or planned use during the study duration
20. Patients likely to be non-compliant to the study procedures or for whom a long-term follow-up seems to be difficult to achieve
21. Relapse that occurs between inclusion and randomization visit

BMRC = British Medical Research Council; EDSS = Expanded Disability Status Scale; TW25 = timed 25-foot walk

Table S2: Primary endpoint – Number of responders (or, and, EDSS only, TW25 only)

Variable	**MD1003** (n=326)	**Placebo** (n=316)
EDSS or TW25, n (%)	39 (12·0)	29 (9·2)
EDSS and TW25, n (%)	5 (1·5)	2 (0·6)
EDSS only, n (%)	22 (6·7)	20 (6·3)
TW25 only, n (%)	22 (6·7)	11 (3·5)

EDSS = Expanded Disability Status Scale; TW25 = timed 25-foot walk

Table S3: Exploratory endpoints (Intention-to-Treat population)

Exploratory endpoints	M15			M27		
	MD1003[#] (n=326)	**Placebo[#]** (n=316)	**one-sided P value**	**MD1003[#]** (n=326)	**Placebo[#]** (n=316)	**one-sided P value**
Change in whole brain volume, mean (SD)[$]	-0.46 (0·55)	-0·49 (0·51)	0·51	-0·53 (0·56)	-0·67 (0·48)	0·21
Change in thalamic volume, mean (SD)[$]	-0·74 (1·35)	-0·74 (1·19)	0·74	-1.10 (1·21)	-0·89 (1·43)	0·68
Change in cortical grey matter volume, mean (SD)[$]	-0·69 (0·72)	-0·74 (0·66)	0·35	-0·97 (0·86)	-1·09 (0·67)	0·27
Change in brain water content as measured by pseudo T2 relaxation time, mean (SD)[$]	0·41 (1·96)	0·016 (2·13)	0·29	1·06 (1·58)	1·61 (1·86)	0·25
Change in the ratio of total N-acetyl groups to creatine (NA/Cr) as measured by MRS, mean (SD)[$]	-0·00 (0·16)	0·01 (0·19)	0·24	-0·01 (0·158)	-0·00 (0·25)	0·56
Change in remote monitoring of ambulatory activity (FitBit®), mean (SD)*	-384·32 (1731·39)	-100·50 (1500·05)	**	-802·12 (1850·29)	-436·19 (1622·82)	n.d.
Change in QoL as measured by MSQoL-54 (patient), PHCS, mean (SD)[$]	-0·48 (11·53)	-1·29 (11·26)	0·28	-0·62 (10·02)	-4·19 (13·47)	0·08
Change in QoL as measured by MSQoL-54 (patient), MHCS, mean (SD)[$]	-1·79 (17·69)	-0·00 (15·28)	0·08	0·50 (15·74)	-6·58 (17·32)	0·03
Change in QoL as measured by CAREQoL-MS (caregiver), Physical Stress/Global Health, mean (SD)[$]	0·07 (0·62)	0·11 (0·59)	0·48	0·29 (0·57)	0·28 (0·66)	0·46
Change in Kurtzke total EDSS score, mean (SD)[$]	0·07 (0·70)	0·08 (0·59)	0·45	0·09 (0·56)	0·90 (0·70)	0·25
Change in SDMT score, mean (SD)[$]	-0·90 (7·14)	-0·80 (7·85)	0·47	-0·70 (6·39)	-1·20 (11·60)	0·48
Change in serum NfL concentration, mean (SD)[£]	0·50 (9·26)	-0·13 (10·13)	[£]	1·83 (5·76)	3·02 (4·35)	n.d.

Subjects at risk: Whole brain volume (Baseline: MD 326; PBO 316; M15: MD 258, PBO 263; M27: MD 52, PBO 42); Thalamic Volume (Baseline: MD 326; PBO 316; M15: MD 256, PBO 259; M27: MD 52, PBO 42); Cortical grey matter volume; (Baseline: MD 326; PBO 316; M15: MD 255, PBO 263; M27: MD 52, PBO 42); Brain water content: (Baseline: MD 145; PBO 140; M15: MD 115, PBO 109; M27: MD 26, PBO 19); MRS, ratio of NA/Cr (Baseline: MD 212; PBO 206; M15: MD 162, PBO 163; M27: MD 33, PBO 27); remote monitoring (FitBit) (Baseline: MD 251; PBO 241; M15: MD 181, PBO 180; M27: MD 38, PBO 35); MSQoL-54 (Baseline: MD 326; PBO 314; M15: MD 284, PBO 281; M27: MD 71, PBO 62); CareQol-MS(Baseline: MD 57; PBO 50; M15: MD 33, PBO 32; M27: MD 14, PBO 5); Kurtzke Total EDSS score (Baseline: MD 323; PBO 316; M15: MD 280, PBO 279; M27: MD 70, PBO 60); SDMT (Baseline: MD 325; PBO 314; M15: MD 271, PBO 276; M27: MD 70, PBO 63); serum NfL (Baseline: MD 297; PBO 287; M15: MD 177, PBO 180; M27: MD 59, PBO 58)

$ One-sided p-value tested with Van Elteren test comparing change from baseline between MD1003 and placebo, stratified for disease history (SPMS/PPMS) and geographical region (N. Am-Aus/Europe)

*Average daily step count is defined as the sum of daily step counts from valid days within a 21 days period prior to the visit divided by the number of valid days (a "valid" day is defined as a day with at least 130 steps); Comparison between Study Treatment Groups at M15: Difference of LS Means at M15 (-224·43 (95%CI) (-510·32, 61·45) The analysis was performed with a MMRM approach for change from baseline in average of daily step counts during 3 weeks prior to each post baseline visit: Fixed effects: study treatment group, geographical region, MS disease history, month of study visit and visit-by-study treatment group interaction.

£ Comparison between Study Treatment Groups at M15: Difference of LS Means at M15 (0.65 (95%CI) (-0·92, 2·23) The analysis was performed with a Mixed Model Repeated Measures approach for absolute change: Fixed effects: study treatment group, geographical region, MS disease history, month of study visit and visit-by-study treatment group interaction.

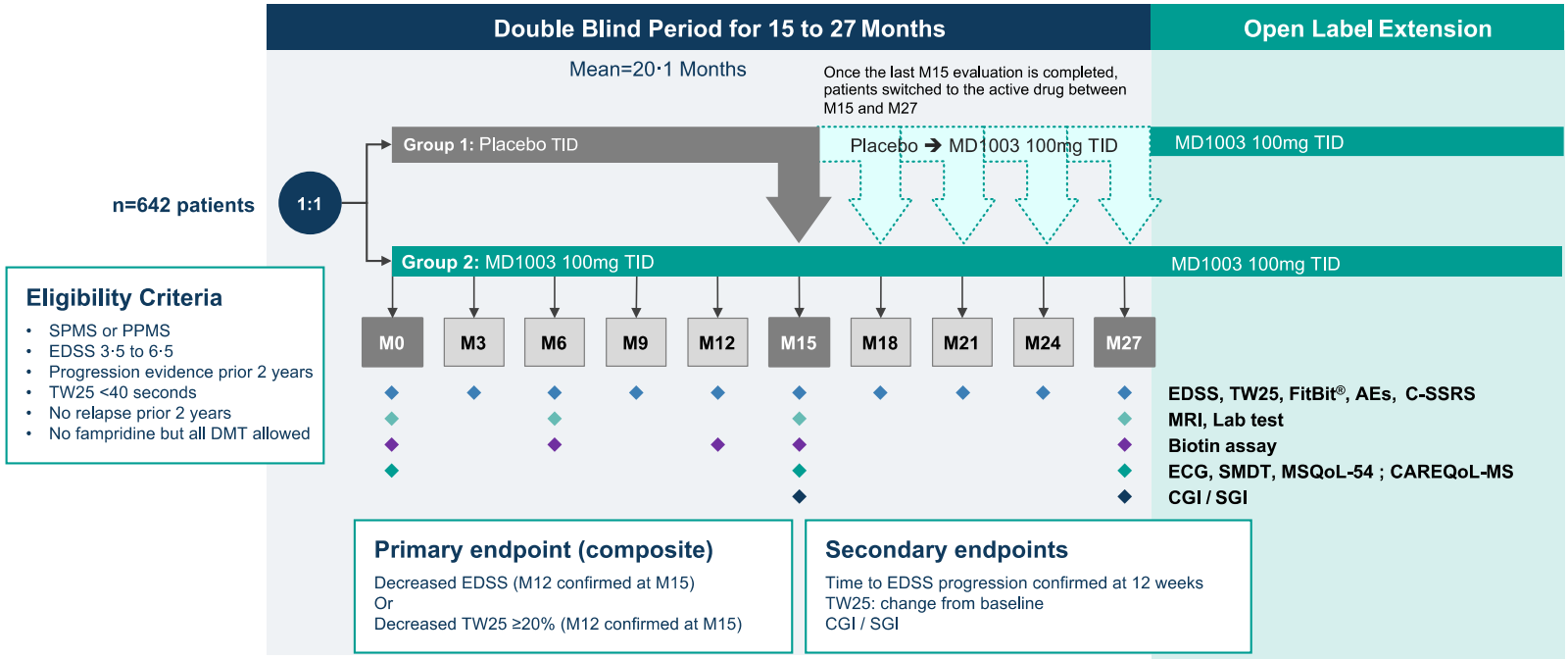
CAREQoL-MS = Caregiver Health-Related Quality of Life in Multiple Sclerosis; EDSS = Expanded Disability Status Score; M = month; MHCS = Mental Health Composite Score; MRS = Magnetic Resonance Spectroscopy; MSQoL-54 = Multiple Sclerosis Quality of Life 54 questionnaire; n = number; n.d. = not determined; PHCS = Physical Health Composite Score; QoL = Quality of Life; SD = standard deviation; SDMT = Symbol Digit Modalities Test; NfL = Neurofilament light chain.

Table S4: Safety – AEs in > 3% of study subjects (Safety population)

	MD1003 (n=331)	**Placebo** (n=311)
AEs in > 3% of subjects		
Urinary tract infection, n (%)	48 (14·5)	50 (16·1)
Nasopharyngitis, n (%)	39 (11·8)	58 (18·6)
Fall, n (%)	44 (13·3)	37 (11·9)
Multiple Sclerosis relapse, n (%)	29 (8·8)	31 (10·0)
Upper respiratory tract infection, n (%)	25 (7·6)	27 (8·7)
Fatigue, n (%)	24 (7·3)	26 (8·4)
Arthralgia, n (%)	25 (7·6)	26 (8·4)
Muscular weakness, n (%)	20 (6·0)	24 (7·7)
Back pain, n (%)	19 (5·7)	21 (6·8)
Gait disturbance, n (%)	22 (6·6)	17 (5·5)
Contusion, n (%)	13 (3·9)	23 (7·4)
Constipation, n (%)	18 (5·4)	16 (5·1)
Depression, n (%)	17 (5·1)	12 (3·9)
Headache, n (%)	13 (3·6)	15 (4·8)
Diarrhoea, n (%)	16 (4·8)	12 (3·9)
Pain in extremity, n (%)	13 (3·9)	13 (4·2)
Laboratory test interference, n (%)	25 (7·6)	0 (0·0)
Muscle spasticity, n (%)	11 (3·3)	14 (4·5)
Multiple Sclerosis, n (%)	8 (2·4)	14 (4·5)
Muscle spasms, n (%)	12 (3·6)	11 (3·5)
Balance disorder, n (%)	11 (3·3)	11 (3·5)
Nausea, n (%)	10 (3·0)	11 (3·5)
Hypoaesthesia, n (%)	10 (3·0)	11 (3·5)

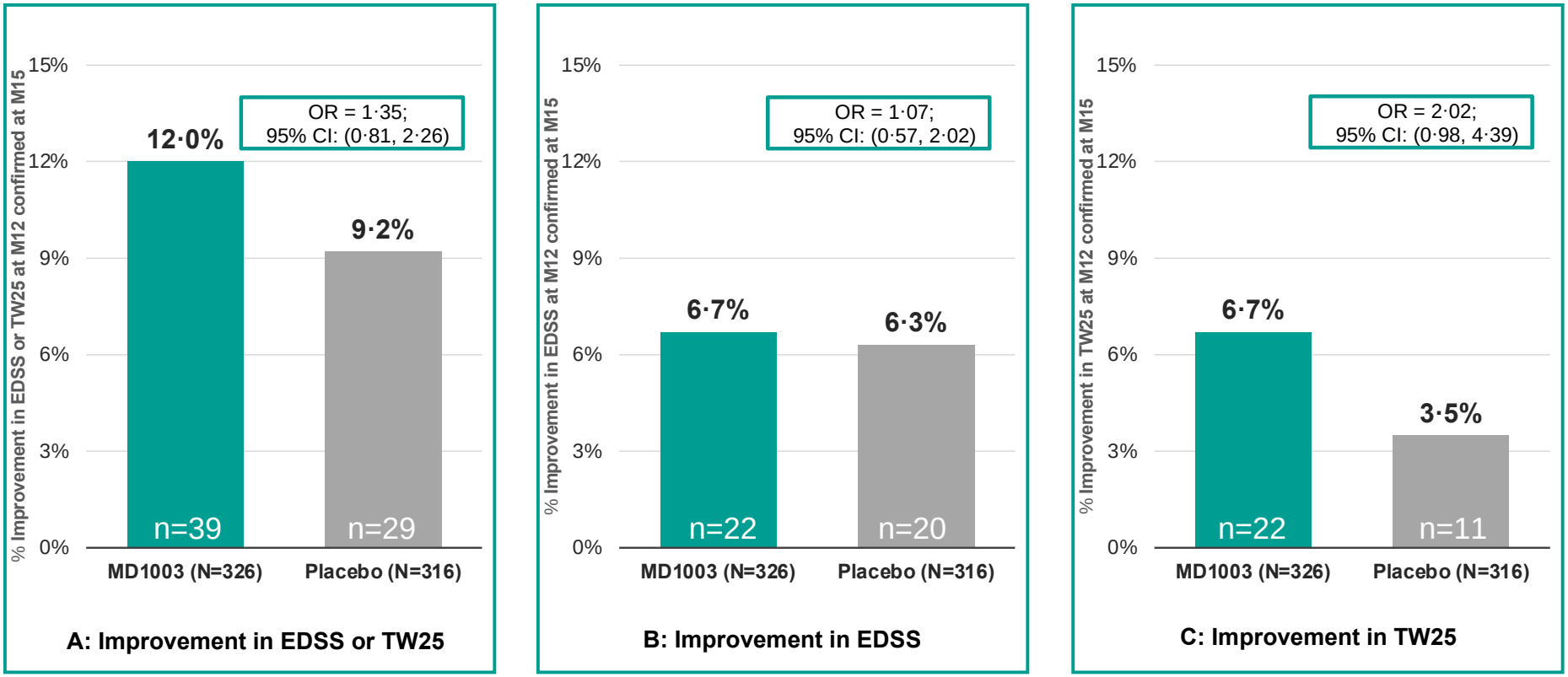
AE = Adverse event

Figure S1 – SPI2 Study Design



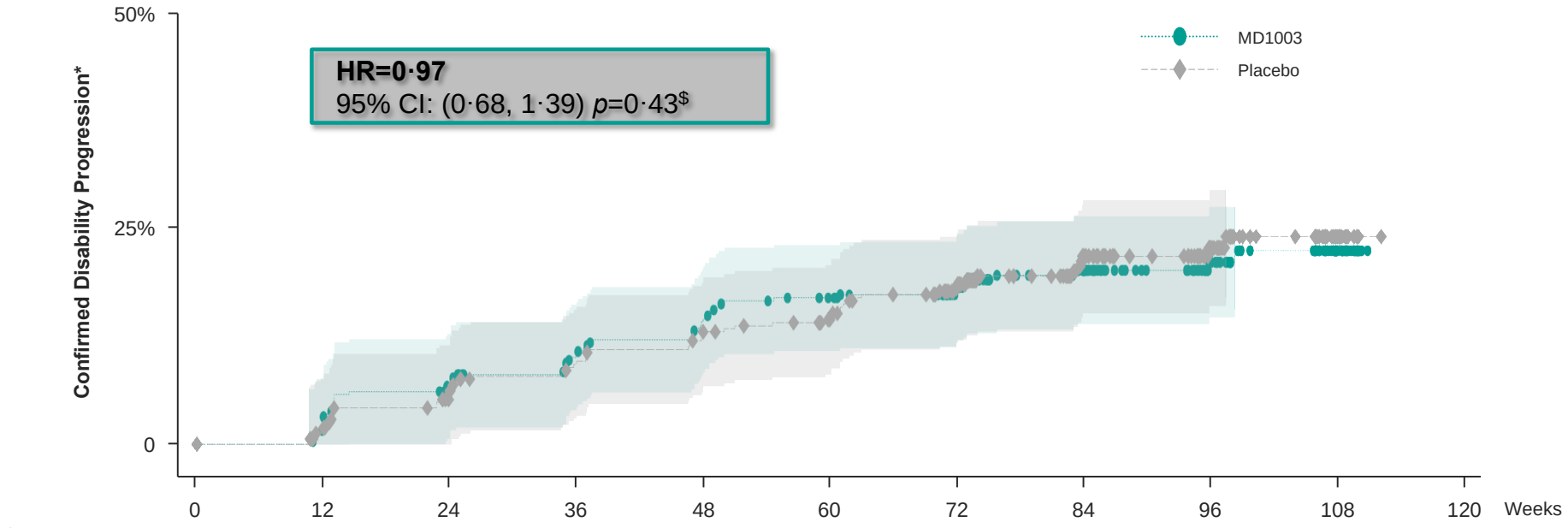
EDSS: Expanded Disability Status Scale; MRS: Magnetic Resonance Spectroscopy; MSQoL-54: Multiple Sclerosis Quality of Life-54; SGI: subject-assessed Clinical Global Impression Scale; SDMT: Symbol Digit Modalities Test; TW25: timed 25-foot walk; Aes: Adverse events; CAREQoL-MS: caregiver health-related quality of life in multiple sclerosis; CGI: clinician-assessed Clinical Global Impression Scale; ECG: electrocardiogram;* ≥ 1 point if initial EDSS from 3·5 to 5·5; ≥ 0.5 point if initial EDSS from 6 to 6·5
Source: Protocol No: MD1003CT2016-01MS-SPI2; EudraCT No: 2016-000700-29

Figure S2: Primary endpoint– Improvement in EDSS or TW25 at M12 confirmed at M15



EDSS response is defined as: decrease of at least 1 point if baseline EDSS 3·5 to 5·5 and of at least 0·5 points if baseline EDSS 6 to 6·5 compared to the lowest EDSS at inclusion and randomization visits.
TW25 response is defined as: decrease of at least 20% compared to the lowest mean of TW25 attempts at inclusion and randomization visits.
Asymptotic logistic regression analysis for the primary efficacy endpoint with study treatment group, disease history, and geographical region as factors; ITT Analysis Set – single imputation of missing values as non-response.
CI: confidence interval; EDSS: Expanded Disability Status Scale; OR: odds ratio; TW25: timed 25-foot walk.

Figure S3: First secondary endpoint – Time to 12-week confirmed EDSS progression



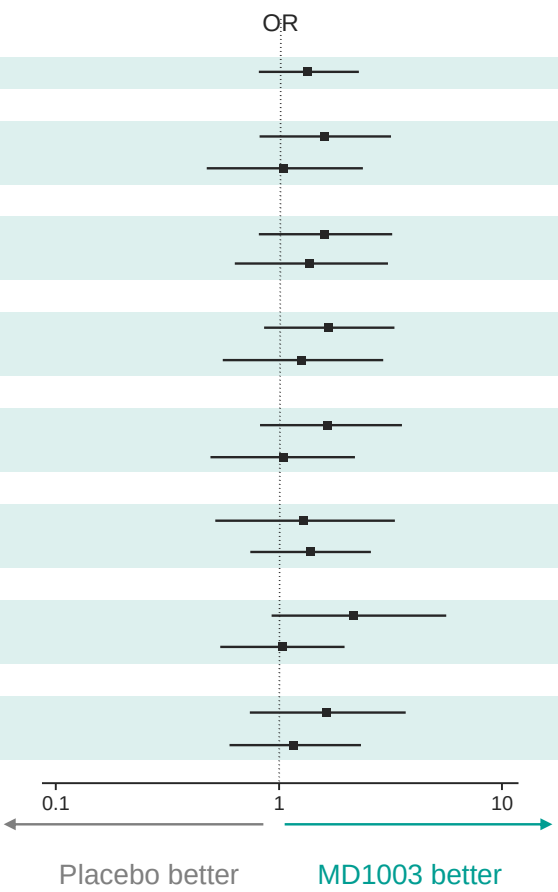
Subjects at risk

MD1003	326	307	284	265	248	233	199	135	85	33
Placebo	316	305	283	264	253	238	208	133	81	26

ITT analysis set; analysis was performed with a Proportional Hazards model stratified for geographical region and disease history. $Wald-test p-value.
*12-week confirmed EDSS progression is defined by an increase of at least 1 point if baseline EDSS 3·5 to 5·5 and of at least 0·5 point if baseline EDSS 6 to 6.5 with respective confirmation 12 weeks later Patients with initial disability progression who discontinued treatment early with no confirmatory EDSS assessment were considered as having confirmed disability progression
EDSS = Expanded Disability Status Scale; HR = hazard ratio; ITT = intention-to-treat

Figure S4 – Subgroup Analysis of Primary Endpoint: Improvement in EDSS or TW25 at M12 Confirmed at M15

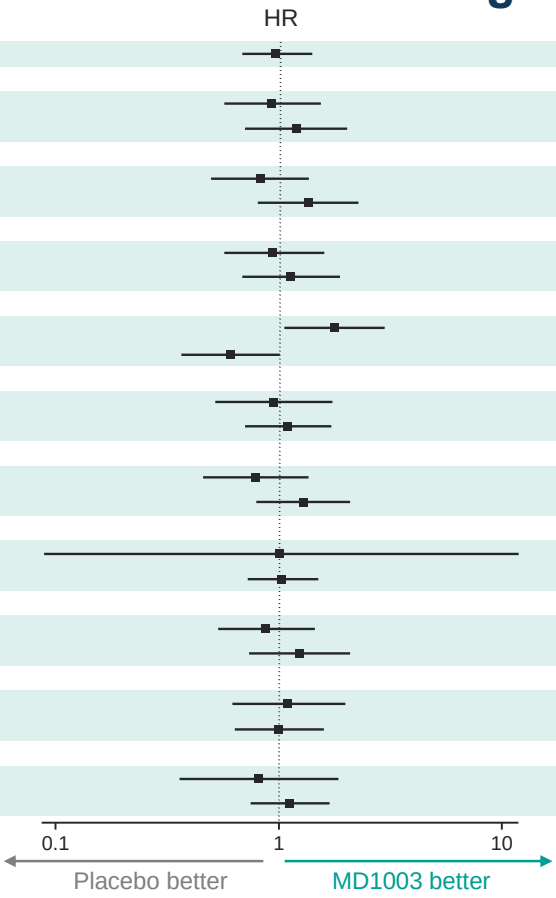
Subgroup	No of Patients (%)	OR	LCL	UCL
Overall	642 (100·0)	1·35	0·81	2·26
Region				
Europe	338 (52·6)	1·59	0·82	3·16
North America - Australia	304 (47·4)	1·05	0·48	2·35
Age				
≤54	353 (55·0)	1·44	0·74	2·87
>54	289 (45·0)	1·23	0·57	2·70
Gender				
Female	345 (53·7)	1·50	0·78	2·93
Male	297 (46·3)	1·14	0·51	2·60
BMI				
≤25·6	319 (49·7)	1·67	0·82	3·53
>25·6	315 (49·1)	1·04	0·50	2·16
Disease History				
Primary Progressive	227 (35·4)	1·28	0·52	3·27
Secondary Progressive	415 (64·6)	1·38	0·75	2·58
EDSS at Baseline				
Up to 5·5	269 (41·9)	2·19	0·93	5·57
6 or above	370 (57·6)	1·04	0·55	1·97
Treated with DMTs at Baseline				
Yes	294 (45·8)	1·62	0·75	3·67
No	348 (54·2)	1·17	0·60	2·31



BMI = body mass index; EDSS = Expanded Disability Status Scale
LCL = lower control limit; OR = odds ratio; UCL = upper control limit

Figure S5 – Subgroup Analysis of First Secondary Endpoint: Time to 12 Week Confirmed EDSS Progression

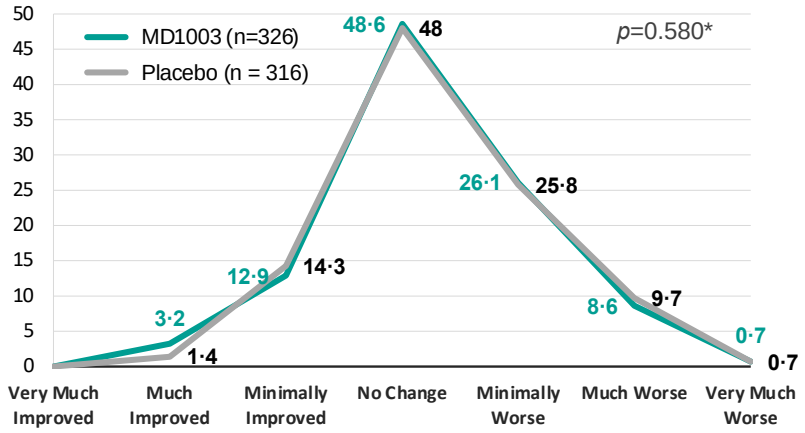
Subgroup	No of Patients (%)	OR	LCL	UCL
Overall	642 (100·0)	0·97	0·68	1·38
Region				
Europe	338 (52·6)	0·92	0·57	1·51
North America - Australia	304 (47·4)	1·18	0·70	1·98
Age				
≤54	353 (55·0)	0·82	0·50	1·34
>54	289 (45·0)	1·34	0v80	2·24
Gender				
Female	345 (53·7)	0·94	0·57	1·56
Male	297 (46·3)	1·12	0·68	1·85
BMI				
≤25·6	319 (49·7)	1·76	1·05	2·95
>25·6	315 (49·1)	0·60	0·36	1·00
Disease History				
Primary Progressive	227 (35·4)	0·94	0·51	1·71
Secondary Progressive	415 (64·6)	1·09	0·70	1·69
EDSS at Baseline				
Up to 5·5	269 (41·9)	0·78	0·45	1·33
6 or above	370 (57·6)	1·27	0·79	2·04
Concomitant Physical Therapy				
Yes	17 (2·6)	1·01	0·06	16·27
No	625 (97·4)	1·03	0·72	1·47
Treated with DMTs at Baseline				
Yes	294 (45·8)	0·87	0·53	1·43
No	348 (54·2)	1·23	0·74	2·05
Myorelaxants at Baseline				
Yes	237 (36·9)	1·09	0·61	1·95
No	405 (63·1)	0·99	0·63	1·55
Rituximab or Ocrelizumab at Baseline				
Yes	90 (14·0)	0·81	0·36	1·82
No	552 (86·0)	1·11	0·75	1·65



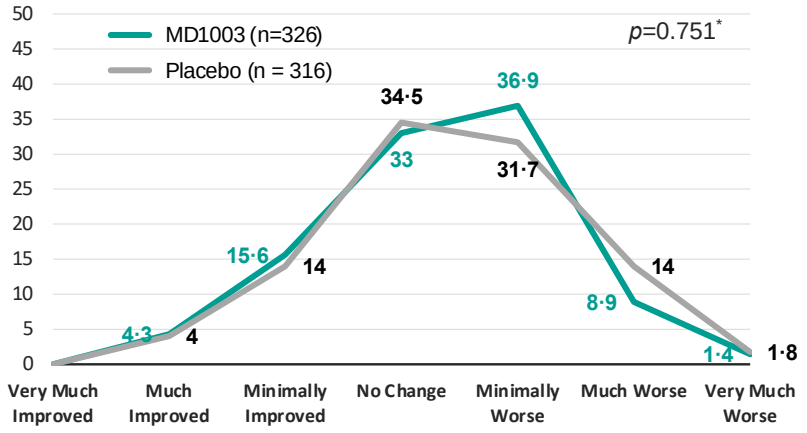
BMI = body mass index; EDSS = Expanded Disability Status Scale
LCL = lower control limit; OR = odds ratio; UCL = upper control limit

Figure S6 – Secondary endpoint – CGI/SGI

Clinician rating (CGI)



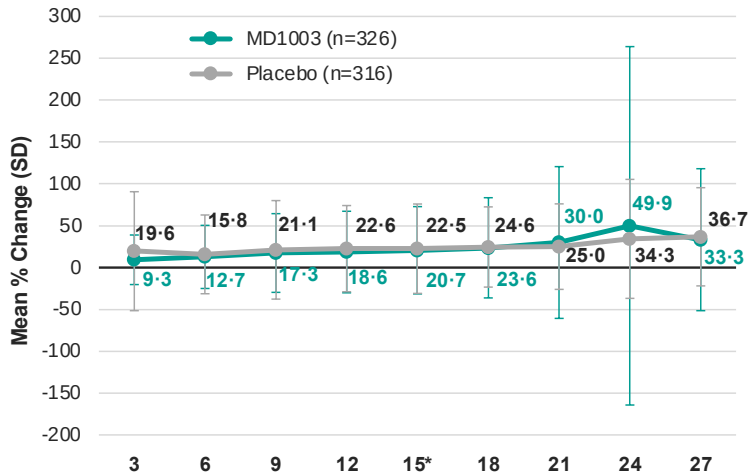
Subject rating (SGI)



ITT analyses set , * One-sided Van Elteren test stratified for disease history (SPMS/PPMS) and geographical region (North America-Australia/Europe) comparing the two study treatment groups

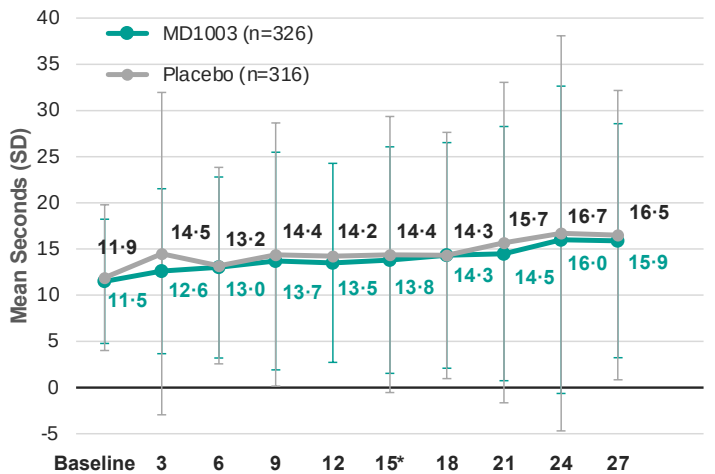
Figure S7 – Secondary Endpoint – Change in T25W

Mean change from Baseline in TW25 % (SD)



Subjects at risk									
MD1003	312	302	294	280	275	257	179	128	67
Placebo	310	294	275	272	270	250	165	116	55

Mean TW25 seconds (SD)



Subjects at risk										
MD1003	324	314	304	290	282	277	259	179	128	67
Placebo	315	311	295	276	273	271	251	165	116	55

*One-sided p=0·5240
Hodges-Lehmann Point estimate for location shift of distributions (95% CI): 0.30 (-5·891 , 6·491)

ITT analyses set; Descriptive summary
*One-sided Van Elteren test stratified for disease history (SPMS/PPMS) and geographical region (North America-Australia/Europe) comparing the two study treatment groups.

Figure S8: Confirmed Relapses by Clinical Adjudication Committee

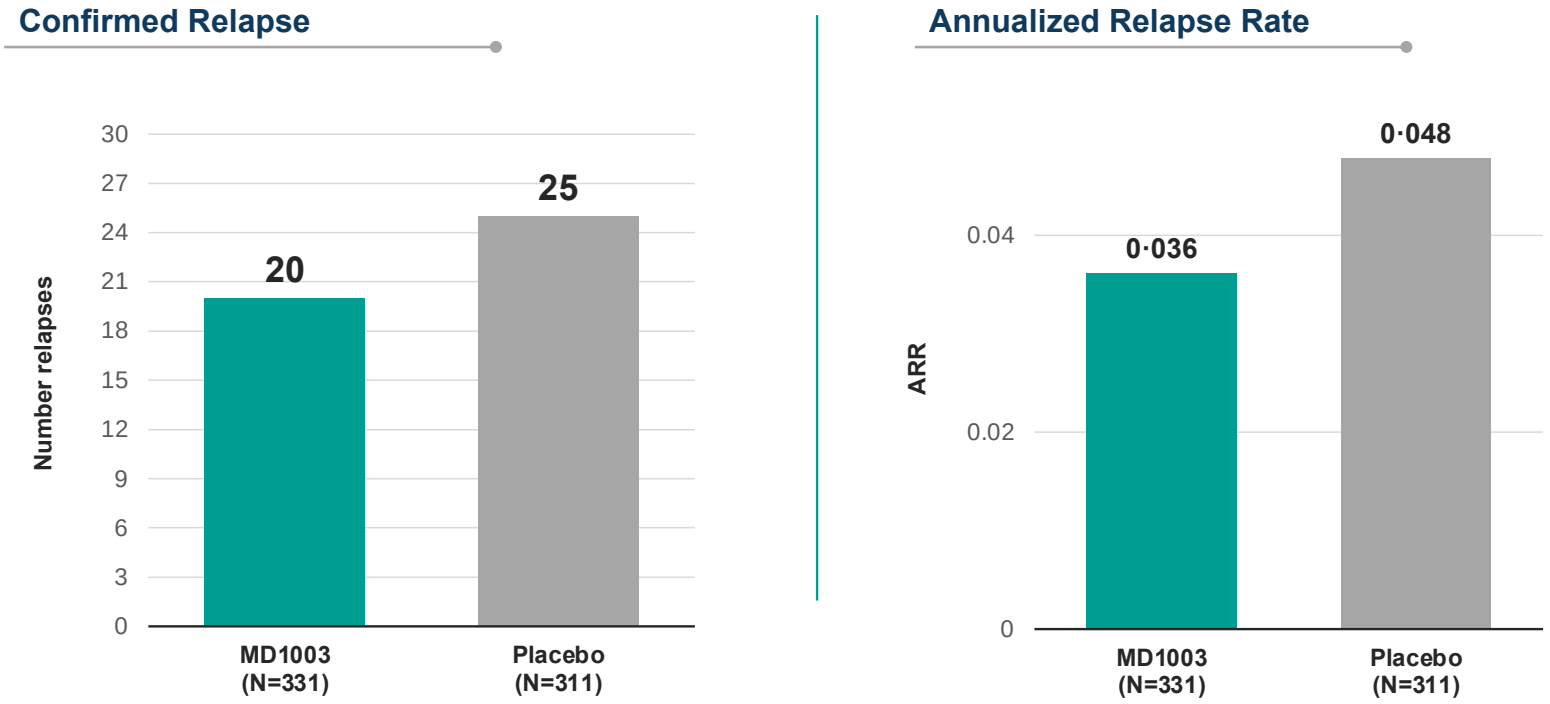
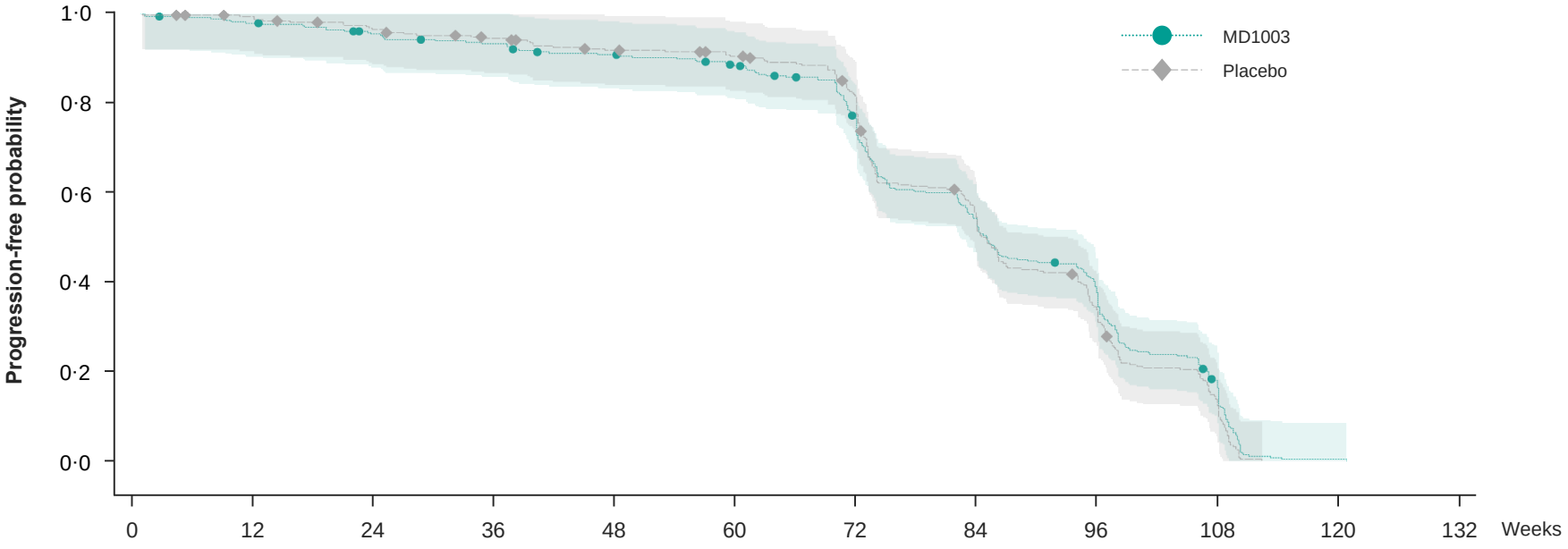


Figure S9: Time to First Relapse Confirmed by the Adjudication Committee

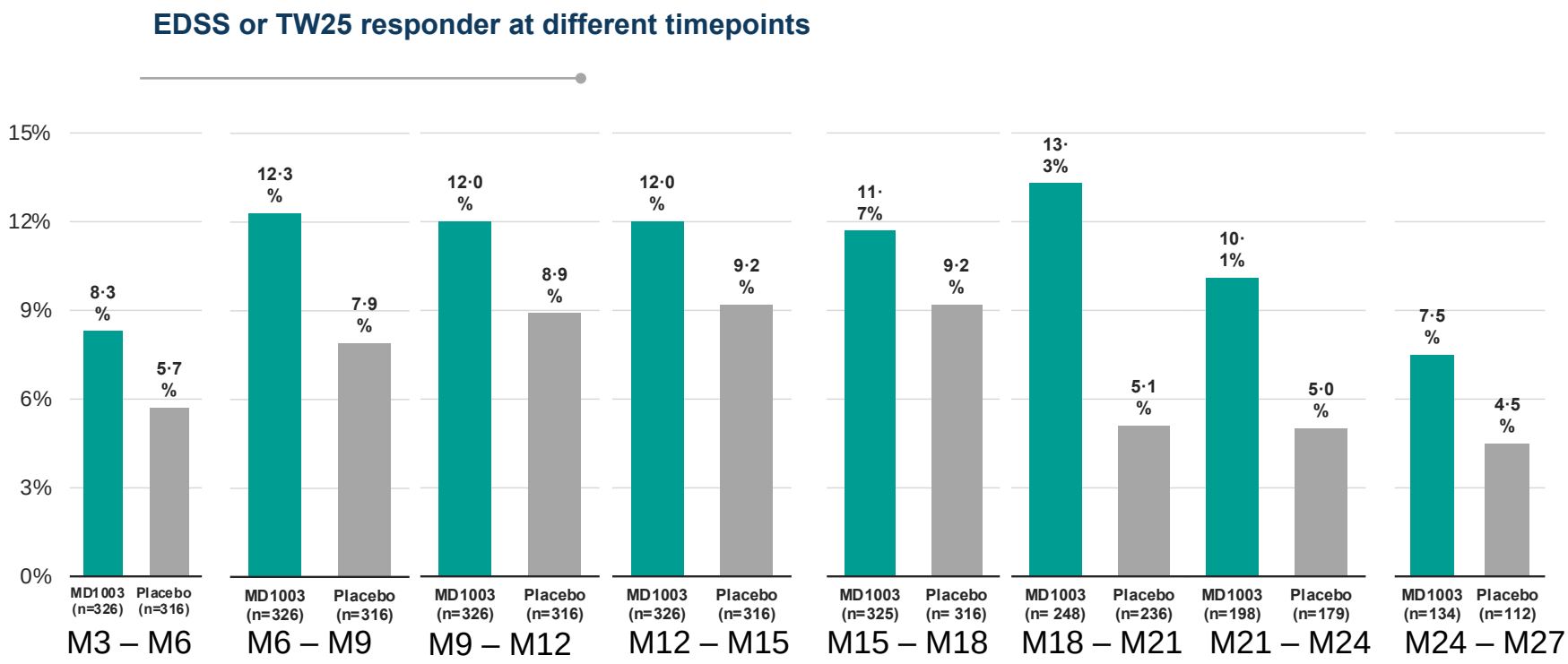


Subjects at risk

MD1003	331	322	311	303	292	281	228	164	107	37	1	0
Placebo	311	306	295	286	275	268	227	149	89	28	0	0

Figure S10: SPI2 Primary Endpoint at Different Timepoints

Improvement in EDSS or Improvement in TW25 confirmed at + 3 months



Additional Descriptive Analysis of Composite Confirmed Response (with Single Imputation of Missing Data as Non-Response) by Visit (ITT Analysis Set)
Response is defined for EDSS: decrease of at least 1 point if baseline EDSS 3·5 to 5·5 and of at least 0·5 point if baseline EDSS 6 to 6·5 compared to the lowest EDSS at inclusion and randomization visits
for TW25: decrease of at least 20% compared to the lowest mean of TW25 attempts at inclusion and randomization visits

Figure S11: Time to Confirmed Disease Improvement on EDSS or TW25

KAPLAN MEIER survival curve

Time to 3 month confirmed disease improvement on EDSS or TW25

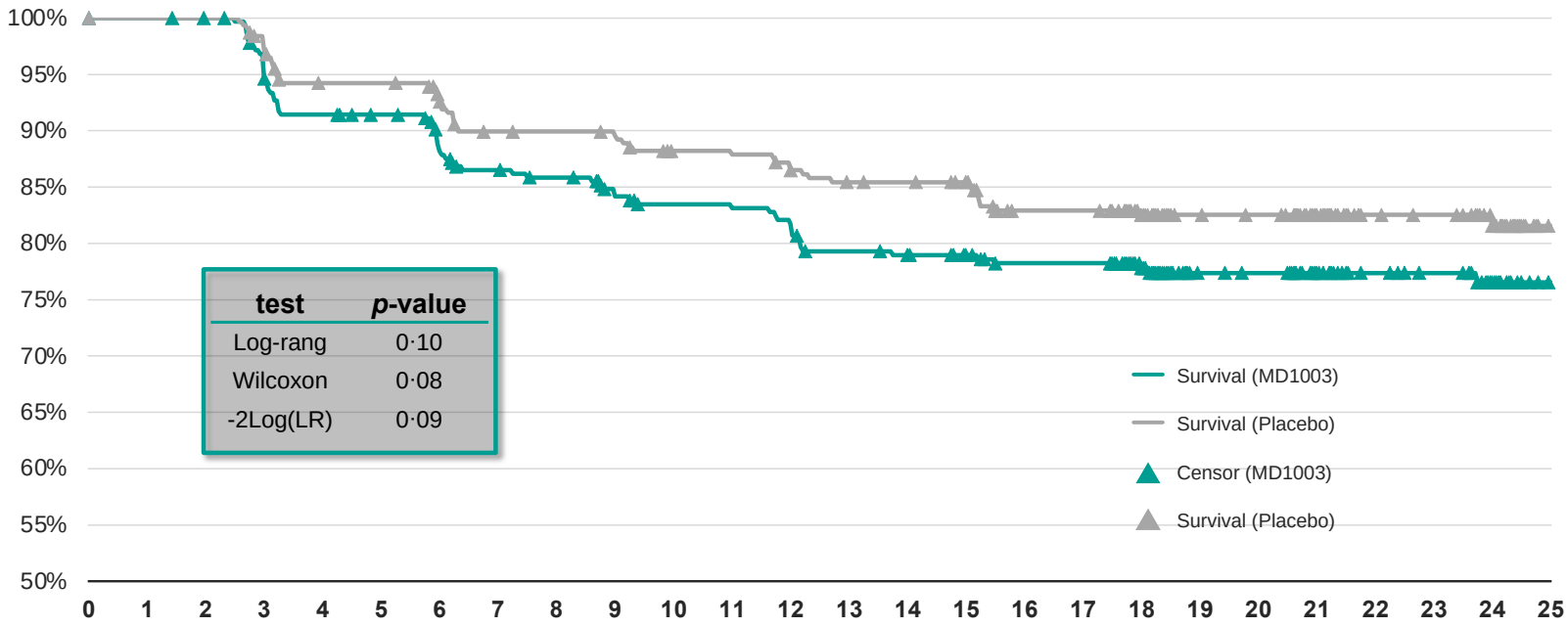
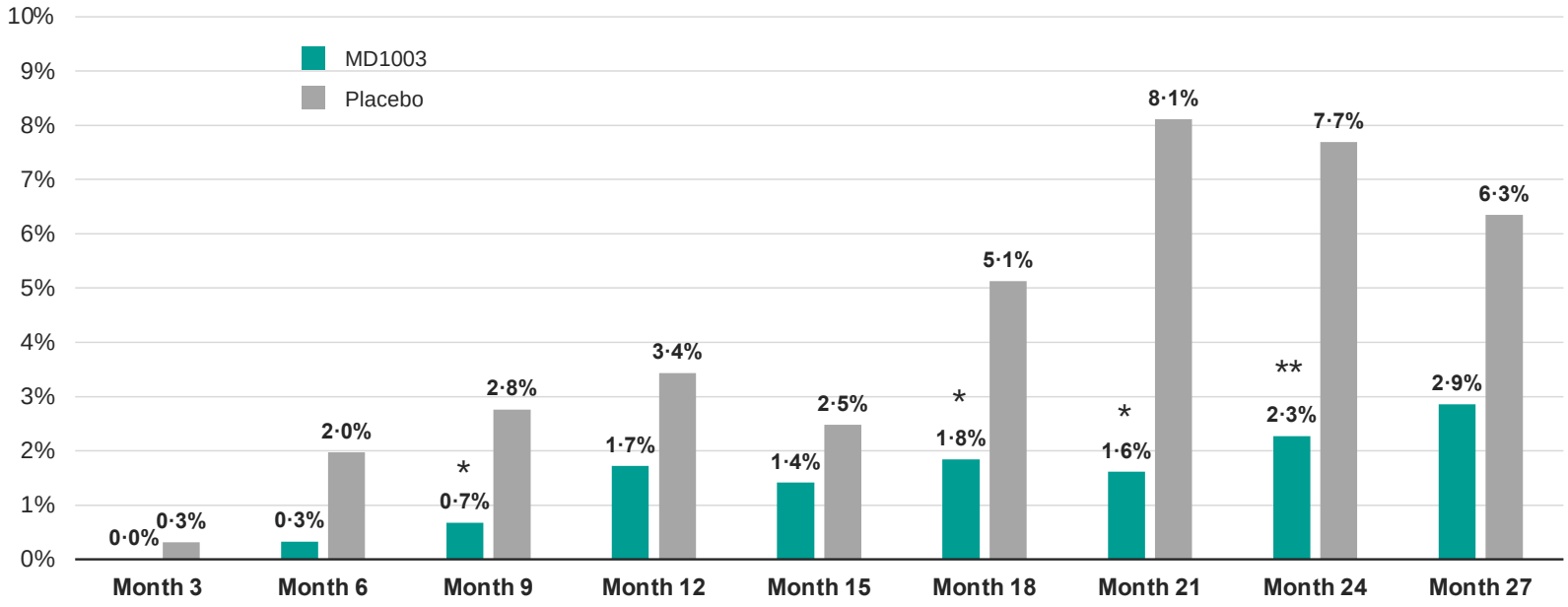


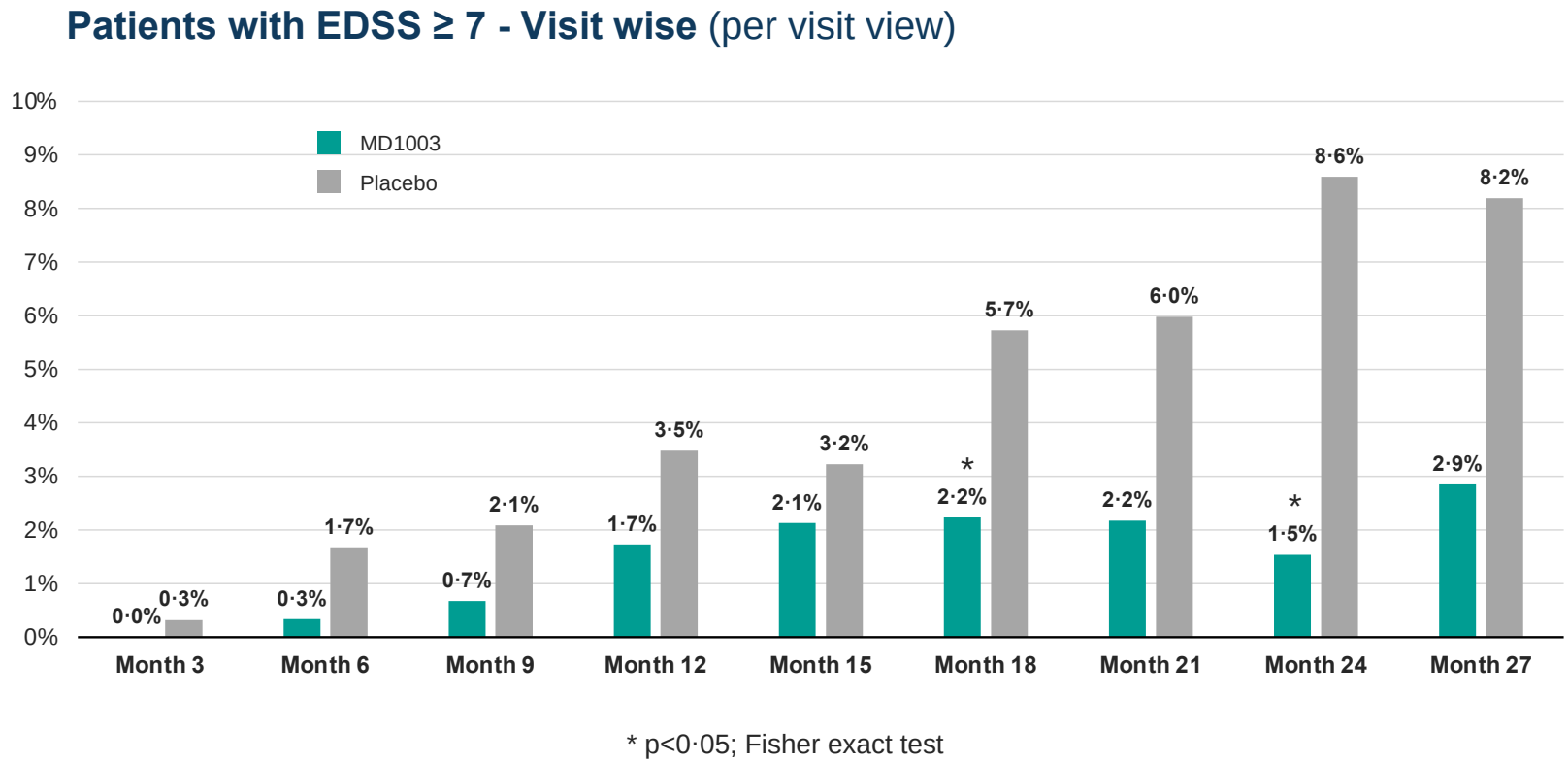
Figure S12: The Proportions of Patients Unable to Perform the TW25 Because of MS Worsening

TW25 missing assessement due to MS worsening (per visit view)



* p<0·05; ** p<0·01, Fisher exact test

Figure S13: The Proportions of Participants with an EDSS of at least 7



* p<0·05; Fisher exact test

Figure S14: EDSS Mean Change versus M0

EDSS mean change *versus* M0 (per visit view)

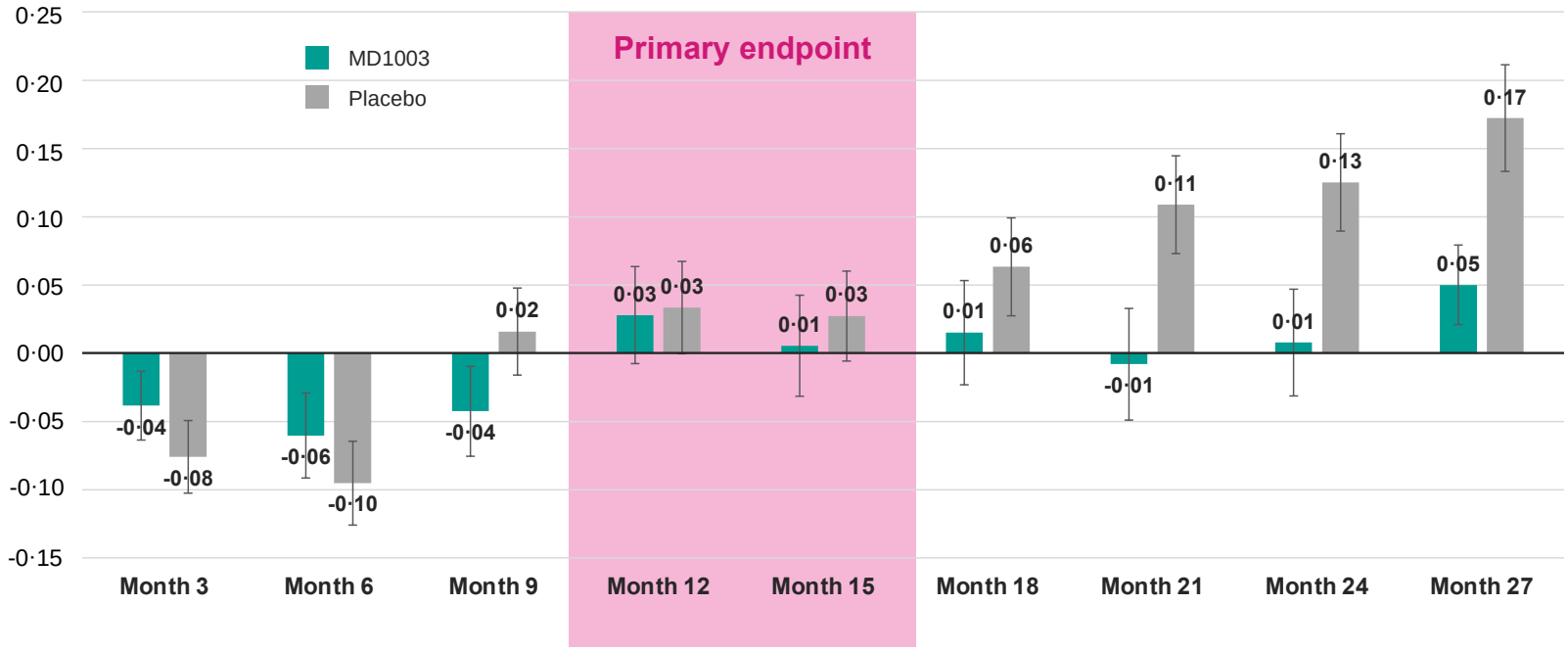


Figure S15: Disability Progression in SPI2, MS-SPI and Other Progressive MS Studies