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Supplementary Information

Supplementary methods

Study design of the PROTYS study

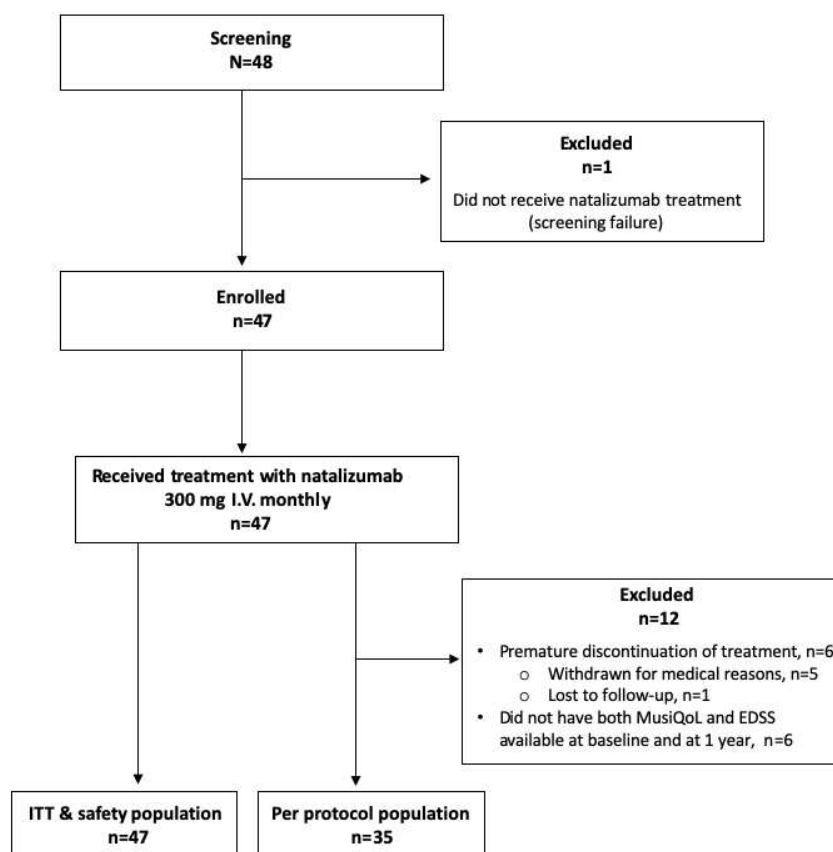
Study design included:

Supplementary Table 1: Inclusion and exclusion criteria for study participation.

Inclusion	Exclusion
<ul style="list-style-type: none"> • Aged 18-65 years old, inclusive, at the time of the informed consent • Able to understand the purpose and risks of the study • Confirmed diagnosis of RRMS, as per the 2010 revised McDonald criteria [1] • EDSS score of 2.0-5.5, inclusive • Natalizumab-naïve patients satisfying the therapeutic indication of natalizumab, as described in the Swiss product label and confirmed by the Investigator <ul style="list-style-type: none"> ○ Decision for treatment with natalizumab was made before screening ○ Patients with previous treatment with natalizumab were also considered to be eligible, if the last natalizumab infusion was at least one year before screening 	<ul style="list-style-type: none"> • Diagnosed co-existing brain pathology other than MS, which in the judgement of the investigator impacts the value of EDSS or QoL • Pure spinal manifestation of demyelination • Diagnosis of primary or secondary progressive MS • Any change in concomitant medication known to affect cognition or bladder function within 4 weeks prior to the baseline visit • A history of severe depressive disorder and/or suicidality, seizure, drug or alcohol abuse, as assessed by the investigator • Insufficient understanding of the local language • Unwillingness or inability to comply with requirements of the protocol, including the presence of any condition (physical, mental or social) that is likely to affect the patient's ability to comply with the protocol

EDSS, Expanded Disability Status Scale; RRMS, relapsing–remitting multiple sclerosis; MS, multiple sclerosis; QoL, quality of life.

Supplementary Figure 1: Study flow diagram.



The intention to treat (ITT) and safety population received at least one infusion of 300 mg natalizumab intravenously (I.V.). The per protocol population were treated with natalizumab for at least one year, received 8 or more natalizumab infusions, and had both Multiple Sclerosis Quality of Life (MusiQoL) and Expanded Disability Status Scale (EDSS) scores available at baseline and at one year.

Secondary objectives of the PROTYS study

Secondary objectives included:

- Assessment of the cumulative probability of sustained disability (EDSS) changes at one year compared to baseline.

- Evaluation of the associations between disability (EDSS), fatigue, sexual dysfunction, depression and neurocognitive function with scores on the Euro-QoL questionnaire (EQ-5D) at three to six-months intervals up to one year compared to baseline.
- Evaluation of the relationship between clinical disease-free status (no confirmed EDSS worsening of ≥ 1.0 point and no relapse) and HRQoL (MusiQoL) at one year compared to baseline.
- Assessment of changes in work impairment at three-month intervals up to one year after initiation of natalizumab treatment.
- Assessment of any change in the percentage of disability pension, work productivity and activity impairment and change in occupation after one year of natalizumab treatment.
- Rates of clinical relapses and relapses requiring steroid treatment at 3-month intervals up to one year after initiation of natalizumab treatment.
- Incidence and number of SAEs during the duration of the study.

Further information on self-administered tests and questionnaires

The MSISQ-19 is a 19-item questionnaire, scored on a scale of 19-95, and designed to assess sexual dysfunction in MS patients. It is divided into three sub-scales: primary (dysfunction due to direct neurological damage involving the genitalia), secondary (dysfunction due to MS symptoms unrelated to the genitalia), and tertiary (dysfunction due to cultural, emotional and psychosocial aspects).[2] The EuroQoL-5D is a standardised questionnaire of health-related quality of life and consists of two parts, index and visual analogue scale (VAS), giving an overall score of health status.[3] The FSMC consists of 20 items on two sub-scales for cognitive and motor fatigue, giving an overall score of 20 (no fatigue) to 100 (severe fatigue).[4] The SDMT is a self-administered test of cognitive processing speed where participants match pairs of symbols and digits over 90 seconds and receive an overall score of processing speed between 0-110.[5] The BDI-FS estimates the severity of depression on a scale of 0-21, with a score of ≥ 4 representing depression.[6] The WPAI-MS

measures impairments in paid and unpaid work due to MS, expressed as percentage impairment.[7]

Supplementary Table 2: HRQoL assessments.

Instrument	No. of items	Domains assessed	Scoring
MusiQoL [8]	31	Physical (8), symptoms (4), psychological (8), self-esteem (4), relationship/friends (3), relationships/family (4)	Total score (0-100) with 0 being worst and 100 best possible level of QoL
MSISQ-19[2]	19	Primary (sexual dysfunction due to direct neurological damage involving genitalia), secondary (sexual dysfunction due to MS symptoms unrelated to the genitalia), tertiary (sexual dysfunction due to cultural, emotional and psychosocial aspects)	Total score(19-95) with higher score indicating more sexual dysfunction
EQ-5D [3]	5	Mobility, self-care, usual activities, pain/discomfort, anxiety/depression	5-digit health state profile describing the patient's health state across the 5 dimensions of health
EQ-VAS [3]	1	Patient's self-rated health	Total score (0-100) with 0 being the worst health you can imagine and 100 being the best health you can imagine
FSMC [4]	20	Cognitive (10) and motor fatigue (10)	Total score (20-100) with 20 being no fatigue and 100 being severe fatigue
SDMT [5]	120 symbols to be paired	Cognitive impairment/disorder	Total score (0-110) with higher scores indicating higher number of correct pairings and better cognitive function
BDI-FS [6]	7	Dysphoria, anhedonia, suicidal ideation, and cognitive-related symptoms	Total score (0-21) with score of ≥ 4 representing depressive symptoms
WPAI-MS [7]	6	Absenteeism, presenteeism, productivity loss and activity impairment	Percent absenteeism, presenteeism, productivity loss due to health, and activity impairment due to health. Higher percentages indicating greater impairment and less productivity

BDI-FS, Beck Depression Inventory-Fast Screen; EQ-5D, EuroQoL-5D; EQ-VAS, EuroQoL-Visual Analogue Scale, FSMC, Fatigue Scale of Motor and Cognitive Function; HRQoL, health-related QoL; MusiQoL, Multiple Sclerosis International QoL; MS, multiple sclerosis; MSISQ-19, MS Intimacy and Sexuality Questionnaire-19; QoL, quality of life; SDMT, Symbol Digit Modalities Test; WPAI-MS, Work Productivity and Activity Impairment in MS.

Post hoc analysis statistical endpoints

For the purpose of this additional *post hoc* analysis, the statistical endpoint is the assessment of the correlation between the following endpoints:

- Change from baseline to EOS in the nine sub-scores of the MusiQoL questionnaire
- Change from baseline to EOS (visit of the last EDSS report) in the EDSS value
- Change from baseline to EOS in the FSMC total score
- Change from baseline to EOS in the WPAI outcomes (absenteeism, presenteeism, productivity loss and activity impairment)
- Change from baseline to EOS in the MSISQ-19 scores (primary, secondary and tertiary causes of sexual dysfunction)
- Change from baseline to EOS in the SDMT score
- Change from baseline to EOS in the EQ-5D index and VAS
- Change from baseline to EOS in the BDI-FS score
- Change from baseline to EOS in the ARR
- Change from baseline to EOS in the ARR of relapses requiring steroid treatment
- Gender, educational level

and the change after 1 year of natalizumab treatment in the global MusiQoL index (categorised as improved or worsened after 1 year), on the complete study population of the per protocol population.

Supplementary results

PROTYS study

Relapses

At one year after initiation of natalizumab treatment, the mean change from baseline in number of relapses requiring corticosteroid treatment was -0.2 ± 1.1 for the EDSS improved group, and -0.4 ± 0.6 for the EDSS stable group.

Post hoc analysis results

Negative or no correlations

Supplementary Table 3: Changes from baseline (BL) to month 12 (EOS).

The results are shown as mean \pm SD. r = non-parametric Spearman's correlation coefficient was used to investigate relationships between the change in the MusiQoL global index at 1 year and the change in the questionnaires scores in each group (improved, worsened), *The changes from baseline to 1 year in the score of each questionnaire were compared between "worsened" or "improved" patients using the Wilcoxon Mann–Whitney test. Negative correlations or associations are bolded.

	MusiQoL Index improved					MusiQoL Index worsened					Difference between MusiQoL groups improved vs. worsened* (p-value)
	n	Baseline	Change from baseline to EOS	correlation		n	Baseline	Change from baseline to EOS	correlation		
				r	p-value				r	p-value	
EDSS score	21	2.83 \pm 1.22	-0.38 \pm 0.84	-0.0877	0.7053	14	3.36 \pm 1.12	-0.25	-0.1847	0.5274	0.5652
ARR - all relapses	20	1.2 \pm 0.7	-0.9 \pm 0.9	0.3385	0.1444	14	0.5 \pm 0.5	-0.5 \pm 0.5	-0.1020	0.7262	0.0653
FSMC total score	21	54.3 \pm 23.2	-3.9 \pm 15.4	-0.6300	0.0022	13	63.2 \pm 24.1	-1.5 \pm 14.3	0.4437	0.1458	1.0000
BDI-FS	15	2.7 \pm 3.2	0.2 \pm 1.4	-0.4930	0.0619	6	2.3 \pm 2.6	0.2 \pm 1.3	-0.1518	0.7741	0.4875
SDMT	21	53.1 \pm 10.6	2.8 \pm 4.9	0.2097	0.3617	14	44.3 \pm 12.6	1.6 \pm 5.3	0.313	0.2759	0.4274
MSISQ-19 primary causes of sexual dysfunction	21	9.7 \pm 5.3	0.6 \pm 4.3	-0.5157	0.0167	14	12.6 \pm 6.0	0.4 \pm 1.7	0.0023	0.9938	0.3780
MSISQ-19 secondary causes of sexual dysfunction	21	17.5 \pm 6.7	-1.1 \pm 4.4	0.0176	0.9395	14	19.6 \pm 6.4	0.6 \pm 4.9	0.0734	0.8030	0.6713

MSISQ-19 tertiary causes of sexual dysfunction	21	9.0±5.3	-1.0±3.7	-0.4113	0.0640	14	10.4±4.9	-0.4±3.0	0.179	0.5404	0.6939
EQ-5D index	21	0.853±0.126	0.024±0.147	0.1935	0.4007	14	0.814±0.184	0.004±0.148	-0.1213	0.6796	0.4893
EQ-5D VAS	21	69.7±19.0	4.8±13.0	0.3511	0.1186	14	71.3±27.6	-1.4±12.6	0.1645	0.5743	0.2497
WPAI presenteeism	12	35.0±32.1	-0.8±39.4	-0.5862	0.0452	4	15.0±19.1	-5.0±10.0	0.2582	0.7418	0.6162

Supplementary Table 3.

See Supplementary Table 3.

- Change in MusiQoL global index and change in EDSS score: No correlation
- Change in FSMC total score and MusiQoL global index: strong negative correlation in “improved” group ($r=-0.6300$, $p=0.0022$)
- Presenteeism score (WPAI) and MusiQoL global index: strong negative correlation in “improved” patients ($r=-0.5862$, $p=0.0452$)
- MSISQ-19 and MusiQoL global index: strong negative correlation in “improved” patients (increase in “primary causes of sexual dysfunction” score, “direct physical causes” score, $r=-0.5157$, $p=0.0167$)
- Negative association between the increase in the “tertiary causes of sexual dysfunction” score and the improvement of the MusiQoL global index (“psychosocial causes” score, $r=-0.4113$, $p=0.0640$)
- Change in SDMT total score and MusiQoL global index: no correlation (both groups)
- Change in EQ-5D index and VAS and MusiQoL global index: no correlation (both groups)
- Change in BDI-FS and MusiQoL global index: moderate negative association in “improved” patients ($r=-0.4930$, $p=0.0619$)

- Change in ARR and MusiQoL global index: no correlation (both groups)

FSMC

A decrease in total fatigue (FSMC score) after one year of treatment with natalizumab was noted in both the “EDSS improved” and “EDSS stable” groups; the decrease was more pronounced in the “EDSS improved” group, although the difference in fatigue scores between the groups was not significant. Additionally, in the *post hoc* analysis, a strong negative correlation between the change from baseline to 1 year in the FSMC total score and the MusiQoL global index was found ($r=-0.6300$, $p=0.0022$) in improved patients. In a larger study on 195 RRMS patients treated with natalizumab for one year,[9] a statistically significant improvement in overall fatigue within one year of natalizumab treatment was also found.[9] It should be noted that in the PROTYS study patients were pre-treated mainly with interferons which could have impacted FSMC scores.

WPAI

Patients with MS are also known to face challenges in their work lives, and work productivity impacts the economic burden of MS.[10] In a previous study, natalizumab treatment was shown to significantly increase work productivity after 50 weeks of treatment.[11] In our study, an improvement in work productivity was seen among the EDSS improved and stable groups within one year of natalizumab treatment. The *post hoc* analysis revealed a strong negative correlation in “MusiQoL improved” patients in the presenteeism score, which reflects an increase in workability. Although these preliminary findings remain to be confirmed in larger studies, this still has importance given patients with low EDSS are already significantly restricted in their ability to work at an age when full working capacity is important.[12]

Supplementary Table 4: Correlation between change in global MusiQoL index after 1 year of natalizumab and the change from baseline to end of study (EOS) in the neurological symptoms and the PROs. The results are shown as mean \pm SD. "Improved": change in the global MusiQoL index >0 , "worsened": change in the global MusiQoL index <0 .

	MusiQoL Index improved		MusiQoL Index worsened	
	Baseline for MusiQoL index improved	Change from Baseline to EOS	Baseline for MusiQoL index worsened	Change from Baseline to EOS
EDSS score	2.83 \pm 1.22	-0.38 \pm 0.84	3.36 \pm 1.12	-0.25 \pm 0
ARR - all relapses	1.2 \pm 0.7	-0.9 \pm 0.9	0.5 \pm 0.5	-0.5 \pm 0.5
ARR - relapses requiring steroid treatment	0.9 \pm 0.7	-0.6 \pm 0.9	0.3 \pm 0.3	-0.2 \pm 0.4
FSMC total score	54.3 \pm 23.2	-3.9 \pm 15.4	63.2 \pm 24.1	-1.5 \pm 14.3
BDI-FS	2.7 \pm 3.2	0.2 \pm 1.4	2.3 \pm 2.6	0.2 \pm 1.3
SDMT	53.1 \pm 10.6	2.8 \pm 4.9	44.3 \pm 12.6	1.6 \pm 5.3
MSISQ-19 primary causes of sexual dysfunction	9.7 \pm 5.3	0.6 \pm 4.3	12.6 \pm 6.0	0.4 \pm 1.7
MSISQ-19 secondary causes of sexual dysfunction	17.5 \pm 6.7	-1.1 \pm 4.4	19.6 \pm 6.4	0.6 \pm 4.9
MSISQ-19 tertiary causes of sexual dysfunction	9.0 \pm 5.3	-1.0 \pm 3.7	10.4 \pm 4.9	-0.4 \pm 3.0
EQ-5D index	0.853 \pm 0.126	0.024 \pm 0.147	0.814 \pm 0.184	0.004 \pm 0.148
EQ-5D VAS	69.7 \pm 19	4.8 \pm 13	71.3 \pm 27.6	-1.4 \pm 12.6
WPAI presenteeism	35 \pm 32.1	-0.8 \pm 39.4	15 \pm 19.1	-5 \pm 10
WPAI absenteeism	21.8 \pm 39.2	-3.2 \pm 33.9	0 \pm 0	0 \pm 0
WPAI productivity loss	46.6 \pm 36.9	-9 \pm 31.2	15 \pm 19.1	-5 \pm 10
WPAI activity impairment	40.8 \pm 27.8	-9.2 \pm 23.1	20 \pm 23.1	0 \pm 16.3

List of abbreviations

ANOVA: one-way analysis of variance

BDI-FS: Beck Depression Inventory

DMT: disease modifying therapy

eCRF: electronic case report form

EDSS: expanded disability status scale

EOS: end of study

EQ-5D: Euro-QoL questionnaire

EQ-VAS: EQ-5D visual analogue scale

FSMC: Fatigue scale of Motor and Cognitive Function

GCP: good clinical practice

HRQoL: health-related quality of life

IV: intravenously

ICH: International Council for Harmonisation

ITT: intention to treat

JCV: John Cunningham virus

LOCF: last observation carried forward

MSIS-29: Multiple Sclerosis Impact Scale

MSISQ-19: Multiple Sclerosis Intimacy and Sexuality Questionnaire-19

MusiQoL; Multiple Sclerosis International Quality of Life questionnaire

RRMS: relapsing-remitting multiple sclerosis

SAE: serious adverse event

SD: standard deviation

SDMT: Symbol Digit Modalities Test

SF-12: Short Form 12

SF-36: Short Form 36

SMSG: Swiss MS Society

VAS: Visual Analogue Scale

WPAI-MS: Work Productivity and Activity Impairment in MS

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