CLINICAL STUDY REPORT

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Study Title: A long-term, non-interventional clinical study to assess the impact of Tysabri® treatment on sleep quality and fatigue in patients with relapsing-remitting multiple sclerosis

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This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and all applicable local regulations.

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS....8
2. PROTOCOL SYNOPSIS....10
3. SUMMARY OF RESULTS AND CONLUSIONS....14
4. ETHICS....15
4.1. Ethics Committees....15
4.2. Patient Information and Consent....15
5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE....16
5.1. Investigators....16
5.2. Study Committees....16
5.3. Vendors....16
6. STATISTICAL ANALYSIS PLAN....17
6.1. Analysis Populations....17
6.2. Statistical Methods for Study Endpoints....17
6.2.1. Null and Alternative Hypotheses....17
6.2.2. Tests of Assumptions....18
6.2.3. Missing Data and Accepted Visit Window....20
6.3. Interim Analyses....20
6.4. Determination of Sample Size....20
7. STUDY SUBJECTS....21
7.1. Subject Accountability....21
7.2. Demographics....25
7.2.1. Distribution of Gender in Patient Population....25
7.2.2. Age Distribution in Patient Population....26
7.3. Baseline Disease Characteristics....27
8. STUDY RESULTS....29
8.1. Primary endpoint – PSQI....29
8.1.1. Descriptive Statistics....29
8.1.2. Hypotheses Testing....33
8.2. Secondary endpoints....34
8.2.1. Impact of the Tysabri® Treatment on Fatigue – MFIS....34
8.2.2. Impact of the Tysabri® treatment on daily sleepiness – ESS....37

8.2.3. Impact of the Tysabri® treatment on subjects' quality of life – EQ-5D 40
8.2.4. Assessment of existence and severity of symptoms of depression – BDI-II....43
8.2.4.1. Results from Hungary and Poland....44
8.2.4.2. Results from Slovakia....46
8.2.5. Occurrence of Clinical Relapses – ARR 48
8.2.6. Assessment of Disability Progression Evaluated by Physician – EDSS 51
8.2.7. Assessment Cognitive Functions – MoCA....54
8.2.8. Impact of the Disease on Subject's Employment....58
8.2.9. Assessment of MRI Changes – T2 Lesions, Gd-enhancing Lesions....61
9. SAFETY RESULTS....64
9.1. Adverse Events....64
9.2. Other assessments....67
9.2.1. JCV....67
9.2.2. NSQ....68
10. DISCUSSION AND OVERALL CONCLUSIONS 81
11. REFERENCE LIST....84
12. APPENDICES....86
APPENDIX A. LISTING OF ETHICS COMMITTEES....87
APPENDIX B. LISTING OF INVESTIGATORS....89
APPENDIX C. STATISTICAL ANALYSIS PLAN....92
APPENDIX D. INVESTIGATOR SIGNATURE PAGE....93

List of Tables

Table 1: List of abbreviations 8
Table 2: Vendors that participated in the study 16
Table 3: Shapiro-Wilk test of normality - PSQI 19
Table 4: Shapiro-Wilk test of normality - PSQI change from baseline on each visit 20
Table 5: Subjects Population – Screened Subjects 21
Table 6: Number of subjects per site 21
Table 7: Subjects disposition 22
Table 8: Reason for withdrawal 25
Table 9: Gender distribution 25
Table 10: Descriptive statistics of age 26
Table 11: Descriptive statistics of baseline disease characteristics 27
Table 12: Number of collected PSQI questionnaires 29
Table 13: Descriptive statistics of PSQI 30
Table 14: Descriptive statistics of PSQI change from baseline on each visit 31
Table 15: Descriptive statistics and ranks for test for several related samples of PSQI (0 - 12 MONTHS) 32
Table 16: Descriptive statistics and ranks for test for several related samples of PSQI (0 - 6 MONTHS) 32
Table 17: Nonparametric one-sample Wilcoxon signed rank test – PSQI change from baseline on visits Month 6 and 12 33
Table 18: Nonparametric Friedman test for several related samples - PSQI on visits Month 0, 6 and 12 33
Table 19: Spearman correlation coefficient - T2 lesions and PSQI on visit Month 0 and 12 33
Table 20: Number of collected MFIS questionnaires 34
Table 21: Modified Fatigue Impact Scale 36
Table 22: Number of collected ESS questionnaires 38
Table 23: Ephworth Sleepiness Scale 38
Table 24: Number of collected EQ-5D questionnaires 40
Table 25: EQ VAS 41
Table 26: EQ-5D-3L 42
Table 27: Number of collected BDI-II questionnaires 43

Table 28: BDI-II in Hungary and Poland 44
Table 29: BDI-II in Slovakia 46
Table 30: Number of subjects with relapses 48
Table 31: Occurrence of ARR 49
Table 32: Number of collected EDSS questionnaires 52
Table 33: EDSS assessed every 6 months 53
Table 34: Number of collected MoCA questionnaires 56
Table 35: MoCA assessment 56
Table 36: Number of employed or economically active patients 58
Table 37: Number of full-time / part-time employed patients 59
Table 38: Last or current employment of patients 60
Table 39: Number of performed MRI examinations every year 61
Table 40: Number of T2-lesions 61
Table 41: Number of Gd-enhancing lesions 63
Table 42: Types of events 65
Table 43: Number of collected JC Virus-detecting tests 67
Table 44: NSQ question #1 – Memory disorders 69
Table 45: NSQ question #2 – Memory recall disorders 70
Table 46: NSQ question #3 – Attention deficit disorders 71
Table 47: NSQ question #4 – Speech sound disorders 72
Table 48: NSQ question #5 –Limb weakness 73
Table 49: NSQ question #6 –Limb coordination disorder 74
Table 50: NSQ question #7 – Walking difficulty 75
Table 51: NSQ question #8 – Visual disorders 76
Table 52: NSQ question #9 – Difficulties with balance 77
Table 53: NSQ question #10 – Frequent change in mood 78
Table 54: NSQ question #11 – Anxiety and depression 79
Table 55: NSQ question #12 – Spasticity of muscles 80

List of Figures

Figure 1: Bar chart of number of subjects per site 22
Figure 2: Bar chart of reasons for withdrawal (n = 80) 25

Figure 3: Pie chart of gender....26
Figure 4: Boxplot of median of age (N = 96)....27
Figure 5: Histogram of CDMS duration at baseline (N = 96)....28
Figure 6: Histogram of last MS attacks at baseline (N = 49)....28
Figure 7: Line graph of mean of PSQI during the study....30
Figure 8: Boxplot of median of PSQI during the study....31
Figure 9: Line graph of mean of MFIS score during the study....36
Figure 10: Boxplot of median of MFIS score during the study....37
Figure 11: Line graph of mean of ESS score during the study....39
Figure 12: Boxplot of median of ESS score during the study....39
Figure 13: Boxplot of median of EQ VAS value during the study....41
Figure 14: Boxplot of median of EQ-5D-3L value during the study....42
Figure 15: Line graph of mean of EQ-5D-3L and EQ-VAS score during the study....43
Figure 16: Boxplot of median of BDI-II score in Hungary and Poland during the study....45
Figure 17: Boxplot of median of BDI-II score in Slovakia during the study....46
Figure 18: Line graph of mean of BDI-II score during the study....47
Figure 19: Bar chart of relapses during the study....49
Figure 20: Bar chart of ARR before and during the study....50
Figure 21: Line graph of mean of EDSS score during the study....53
Figure 22: Boxplot of median of EDSS value during the study....54
Figure 23: Line chart of mean of MoCA during the study....57
Figure 24: Boxplot of median of MoCA test during the study....57
Figure 25: Bar chart of employed or economically active patients....58
Figure 26: Bar chart of full-time / part-time employed patients....59
Figure 27: Bar chart of last or current employment of patients....60
Figure 28: Line graph of mean of T2 lesions during the study....62
Figure 29: Boxplot of median of number of T2 lesions during the study....62
Figure 30: Bar chart of number of active Gd-enhancing lesions during the study....63
Figure 31: Bar chart of type of event....66
Figure 32: Number of positive/negative JC Virus-detecting tests....67
Figure 33: Frequency of positive answer in NSQ....68
Figure 34: Bar chart of NSQ question #1 - Memory disorders....69

Figure 35: Bar chart of NSQ question #2 - Memory recall disorders....70
Figure 36: Bar chart of NSQ question #3 – Attention deficit disorders....71
Figure 37: Bar chart of NSQ question #4 – Speech sound disorders....72
Figure 38: Bar chart of NSQ question #5 – Limb weakness....73
Figure 39: Bar chart of NSQ question #6 – Limb coordination disorder....74
Figure 40: Bar chart of NSQ question #7 – Walking difficulty....75
Figure 41: Bar chart of NSQ question #8 - Visual disorders....76
Figure 42: Bar chart of NSQ question #9 – Difficulties with balance....77
Figure 43: Bar chart of NSQ question #10 – Frequent change in mood....78
Figure 44: Bar chart of NSQ question #11 – Anxiety and depression....79
Figure 45: Bar chart of NSQ question #12 – Spasticity of muscles....80

1. LIST OF ABBREVIATIONS

Table 1: List of abbreviations

AE	Adverse Event
ANOVA	Analysis Of Variance
ARR	Annualized Relapse Rate
BDI-II	Beck Depression Inventory II
CDMS	Clinically Definite Multiple Sclerosis
CI	Confidence Interval
CRF	Case Report Form
Df	Degrees of Freedom
EC	Ethics Committee
EDSS	Kurtzke Expanded Disability Status Scale
EOS	End Of Study
EQ-5D-3L	EuroQoL Questionnaire with 5 dimensions and 3 levels
ESS	Ephworth Sleepiness Scale
EU	European Union
Gd	Gadolinium
ICF	Informed Consent Form
JCV	John Cunningham Virus
MFIS	Modified Fatigue Impact Scale
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
N	Total Number of Subjects
n	Number of Subjects in Each Subgroup
NSQ	Neurological Symptoms Questionnaire
PML	Progressive multifocal leukoencephalopathy
PSQI	Pittsburgh Sleep Quality Index
QoL	Quality of Life
RRMS	Relapsing-Remitting Multiple Sclerosis
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SD	Standard Deviation
SPC	Summary of Product Characteristics
UNB	University Hospital Bratislava (Univerzitná nemocnica Bratislava)
VAS	Visual Analogue Scale

2. PROTOCOL SYNOPSIS

STUDY TITLE	A long-term, non-interventional clinical study to assess the impact of the Tysabri® treatment on sleep quality and fatigue in patients with relapsing-remitting multiple sclerosis
PROTOCOL NUMBER/ VERSION	SLK-TYS-11-10230
SPONSOR	Biogen Idec
STUDY PHASE	Non-interventional clinical study
STUDY SITES	Totally, 24 neurology care sites in Slovakia (5), Hungary (7) and Poland (12) specialized in multiple sclerosis (MS) diagnosis, therapy and long-term follow-up observation were initiated in study.
ENROLLMENT PERIOD	24 months
STUDY PERIOD (FIRST ENROLLMENT – LAST SUBJECT OUT)	5 years
STUDY DRUG	Tysabri® (natalizumab)
STUDY DESIGN	Prospective, observational, multicenter, non-interventional clinical study
STUDY OBJECTIVES	**Primary objective:** • To assess the impact of the Tysabri® treatment on sleep quality (Pittsburgh Sleep Quality Index - PSQI) **Secondary objectives:**

	• To assess the impact of the Tysabri® treatment on fatigue (Modified Fatigue Impact Scale -MFIS) • To assess the impact of the Tysabri® treatment on daily sleepiness (Epworth Sleepiness Scale – ESS) • Impact of the Tysabri® treatment on subject's quality of life (EuroQoL Questionnaire with 5 dimensions - EQ-5D) • To assess existence and severity of symptoms of depression (Beck Depression Inventory II – BDI-II) • Occurrence of clinical relapses (Annualized Relapse Rate – ARR) • To assess disability progression (Kurtzke Expanded Disability Status Scale – EDSS) evaluated by physician • To assess cognitive functions (Montreal Cognitive Assessment – MoCA) • To assess the impact of the disease on subject's employment • To assess changes in magnetic resonance imaging - MRI (T2 lesions, gadolinium-enhancing [Gd-enhancing] lesions) • A long-term safety assessment of the Tysabri® treatment (adverse events – AEs, John Cunningham virus – JCV, neurological symptoms questionnaire – NSQ)
PLANNED NUMBER OF SUBJECTS	Approximately 200 subjects will be enrolled within 2 years

INCLUSION CRITERIA	1. Signed written informed consent 2. 18 ≥ 65 years of age 3. PSQI ≥ 5 4. Diagnosis of relapsing-remitting multiple sclerosis (RRMS) documented clinically and laboratory 5. EDSS ≤ 4 6. Tysabri® naïve (beginning of Tysabri® medication according to medical guidelines (Summary of Product Characteristics - SPC) no earlier than 1 month prior to enrolment) 7. Subject with disease activity despite treatment with beta-interferon or glatiramer acetate. 8. Decision to treat with Tysabri® proceeds consent for the study.
EXCLUSION CRITERIA	1. Progressive multifocal leukoencephalopathy (PML) 2. Increased risk for opportunistic infections 3. Immunocompromised state caused by current or prior immunosuppressive therapy 4. Combination with beta-interferon or glatiramer acetate 5. Known active malignancies, except for patients with cutaneous basal cell carcinoma 6. Pregnancy or breastfeeding 7. Legal incapability or limited legal capability 8. Participation in any other clinical study 9. Medical or psychological condition which in the opinion of the investigator would not permit the patient to complete the study or sign meaningful informed consent

DOSING REGIMEN	Tysabri® 300mg IV every 4 weeks
STUDY ENDPOINTS	**Primary endpoint:** • Sleep quality (PSQI score) **Secondary endpoints:** • Fatigue (MFIS score) • Daily sleepiness (ESS score) • Existence and severity of symptoms of depression (BDI-II) • Cognitive functions (MoCA score) • Relapse occurrence (ARR) • Disability progression (EDSS score) • MRI changes (number of T2, Gd-enhancing lesions) • Quality of life (EQ-5D score) **Safety endpoints:** • Safety information regarding adverse events (AEs), serious adverse events (SAEs) and John Cunningham virus (JCV) positivity
STATISTICAL METHODS	Continuous variables will be summarized with descriptive statistics (number - N, Mean, Median, Minimum, Maximum and Standard deviation). Discrete variables will be presented with frequency distributions (N, %). Statistical analyses for selected continuous variables will be done using a paired t-test or one way analysis of variance (ANOVA). Dependence of global PSQI score and continuous variables will be analyzed using the linear regression model or by means of Spearman correlation coefficients.

3. SUMMARY OF RESULTS AND CONLUSIONS

Number of Subjects (Planned and Analyzed):

The study projection was for 200 subjects to participate in the study; 137 subjects were screened and 96 were successfully enrolled together in all precipitating countries (although end of study visit was available in 91 patients as 5 withdrawn patients had unavailable last page of CRF).

Criteria for Evaluation:

- PSQI
- MFIS
- ESS
- EQ-5D
- BDI-II
- ARR
- EDSS
- MoCA
- information on subjects' employment
- number of T2 lesions, Gd-enhancing lesions
- AE
- anti-JCV antibodies
- NSQ

Results:

- Significant PSQI change (positive effect on sleep quality) from baseline was confirmed between visit in Month 6 [(P = 0.004, n = 67, 95% CI (-1.76 to -0.36)], however differences in PSQI among baseline, Month 6 and Month 12 were not significant. Due to low number of cases (< 30), the tests were omitted at visits Month 18, 24, 30 and 36 as the results could be irrelevant.
- Improvement was observed in MFIS, ESS, ARR, Gd-enhancing lesions and to month 24 in BDI-II. Deterioration was observed in EDSS and T2 lesions and no change was in EQ-5D, EQ-VAS (Visual Analogue Scale) and MoCA test.
- There were 62 % economically active subjects at baseline and 51 % at end of study. Part-time resp. full-time employment was observed in 18 % resp. 83 % at baseline and in 19 % resp. 81 % at end of study. Manual resp. intellectual employment was recorded in 27 % reps. 73 % at baseline and in 20 % resp. 80 % at end of study.
- There were recorded 104 events (AE 72%, SAE 27 %). The most frequent events were Nervous system disorders (36%), then Respiratory, thoracic and mediastinal disorders (17%) and Musculoskeletal and connective tissue disorders (7%)..
- JC Virus positivity mildly increased in third year of observation (month 36). In NSQ, there was determined the most difficulty with limb weakness, walking, balance, and spasticity.

Conclusion(s): Although, some of the results could be influenced by small number of patients and especially those, who completed the latest visits in the study, benefits of Tysabri® treatment were confirmed in the present study.

4. ETHICS

4.1. Ethics Committees

This study is non-interventional, and therefore falls outside the scope of the European Union (EU) Directive 2001/20/EC and the EU Directive 2005/28/EC.

As required by applicable local regulations, all legal regulatory aspects were covered during the study conduct, and approvals from the appropriate regulatory bodies and the independent ethic committees (EC) prior to study initiation were obtained. This study adhered to all local regulatory requirements applicable to non-interventional studies.

In Slovakia, the approval from both the competent authority and EC is not requested for non-interventional study; however, submission to multicenter EC and local ECs for each site was performed despite this fact. In Hungary, the regulatory authority submitted study to EC as part of its own approval. Similarly, favorable opinion of multicenter EC was a component of regulatory authority notification in Poland, although it is not required by Polish law. A list of the ECs for the sites participating in the study is included in Appendix A.

Furthermore, the investigators were responsible for ensuring that the study was performed in accordance with the protocol, the Declaration of Helsinki and applicable regulatory requirements.

4.2. Patient Information and Consent

Written informed consent was obtained from each subject prior any evaluation performed for eligibility. Subjects were given adequate time to review the information in the informed consent and were allowed to ask, and have answered, questions concerning all portions of the conduct of the study. Subjects were provided with a copy of the signed and dated informed consent form (ICF).

The first patient signed ICF on 3rd April 2012 and the last patient was enrolled on 23rd November 2015.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

5.1. Investigators

The study was performed as a multicenter study at 24 investigational sites in Slovakia (5 sites), Hungary (7 sites) and Poland (12 sites). A Principal Investigator (PI) at each site was responsible for the conduct of the study at that site. Prof. MUDr. Peter Turčáni, PhD. acted as the Scientific Coordinator for all sites. A list of all Investigators and their affiliations are provided in Appendix B. Investigators' curricula vitae are available upon request.

5.2. Study Committees

No study committee was held in the study therefore this section is not applicable.

5.3. Vendors

A list of vendors and contractors that had responsibilities for study conduct, and data handling, analysis, and reporting are presented in Table 2.

Table 2: Vendors that participated in the study

Vendor Name	Vendor Address	Responsibilities
Neox s.r.o.	V Jámě 1, 110 00 Praha 1, Czech Republic	Monitoring, data management and analysis, medical writing
Transmedic Slovakia s.r.o.	Lazovná 68, 974 01 Banská Bystrica, Slovakia	Safety reporting
CPS Cortex Gyógyszeripari Szolgáltató Kft	József nádor tér 5-6. III. em., H-1051 Budapest, Hungary	Monitoring
Vigiland Pharma Kft	Bartók Béla út 105-113., 2. ép. 1. em./2., H-1115 Budapest, Hungary	Safety reporting
ClinTec International Sp. z o.o.	ul. Retoryka 1/14 31 -108 Kraków, Poland	Monitoring
Regulatory Affairs Doradztwo, K.Sabiłło & Wspólnicy Sp. j.	ul. Puławska 111a lok. 67, 02-707 Warszawa, Poland	Safety reporting

6. STATISTICAL ANALYSIS PLAN

6.1. Analysis Populations

The full analysis set to assess effectiveness includes all subjects (irrespective of follow up). Eligible subjects must meet all inclusion and no exclusion criteria and must be treated by with Tysabri® at least once within the study.

No safety population is defined for this study.

6.2. Statistical Methods for Study Endpoints

Continuous variables are summarized with descriptive statistics (N, Mean, Median, Minimum, Maximum and Standard deviation). Discrete variables are presented with frequency distributions (N, %).

Statistical analyses for selected continuous variables was planned to be done using a paired t-test or one way analysis of variance (ANOVA). In the case that the data distribution shows to be significantly different from normal distribution, nonparametric versions of the tests, namely Mann-Whitney U test or Kruskal-Wallis test, would be used. Discrete variables was planned to be analyzed using contingency tables and McNemar's tests. All testing used two-sided tests with the criteria set at $\alpha = 0.05$.

Dependence of global PSQI score and continuous variables was analyzed using the linear regression model or by means of Spearman correlation coefficients.

All results (including p values of tests) are presented in a descriptive manner in the form of graphs and tables.

6.2.1. Null and Alternative Hypotheses

Hypotheses – one-sample tests:

H0: Change from Baseline is equal to 0.

H1: PSQI (6 MONTHS – 0 MONTHS) is not equal to 0.

H2: PSQI (12 MONTHS – 0 MONTHS) is not equal to 0.

H3: PSQI (18 MONTHS – 0 MONTHS) is not equal to 0.

H4: PSQI (24 MONTHS – 0 MONTHS) is not equal to 0.

H5: PSQI (30 MONTHS – 0 MONTHS) is not equal to 0.

H6: PSQI (36 MONTHS – 0 MONTHS) is not equal to 0.

Hypotheses – ANOVA / Friedman test:

H0: There are no differences between PSQI on particular visits.

H7: PSQI on one or more visits (0, 6, 12, 18, 24, 30, 36 MONTHS) is different than other.

H8: PSQI on one or more visits (0, 6, 12, 18, 24, 30 MONTHS) is different than other.

H9: PSQI on one or more visits (0, 6, 12, 18, 24 MONTHS) different than other.

H10: PSQI on one or more visits (0, 6, 12, 18 MONTHS) is different than other.

H11: PSQI on one or more visits (0, 6, 12 MONTHS) is different than other.

H12: PSQI on visit 6 MONTHS is not equal to PSQI on Baseline.

Hypotheses – correlation between PSQI and number of MRI T2 lesions:

H0: PSQI on particular visit does not depend on number of T2 lesions.

H13: PSQI on Baseline depends on number of T2 lesions on Baseline.

H14: PSQI on 12 MONTHS depends on number of T2 lesions on 12 MONTHS.

H15: PSQI on 24 MONTHS depends on number of T2 lesions on 24 MONTHS.

H16: PSQI on 36 MONTHS depends on number of T2 lesions on 36 MONTHS

6.2.2. Tests of Assumptions

Shapiro-Wilk test was used for assumption of normality. The alpha level for hypotheses testing is 0.05. P-value less or equal to alpha level 0.05 indicates statistical significance of alternative hypothesis (e.g. not normal distribution).

The PSQI does not have normal distribution on each particular visit hence nonparametric tests like Wilcoxon Signed Rank test, Friedman test and Spearman correlation coefficient was used for hypotheses testing. In Table 3 and

Table 4, distribution of PSQI and change from baseline respectively are analyzed by Shapiro-Wilk test.

Table 3: Shapiro-Wilk test of normality - PSQI

	Shapiro-Wilk test			
	Statistic	**Df**	**p-value**	**Distribution**
PSQI (0 MONTHS)	0.744	96	< 0.001	*not normal distribution*
PSQI (6 MONTHS)	0.909	67	< 0.001	*not normal distribution*
PSQI (12 MONTHS)	0.942	52	0.013	*not normal distribution*
PSQI (18 MONTHS)	0.820	29	< 0.001	*not normal distribution*
PSQI (24 MONTHS)	0.921	20	0.103	*normal distribution*
PSQI (30 MONTHS)	0.893	12	0.127	*normal distribution*
PSQI (36 MONTHS)	0.900	9	0.250	*normal distribution*

Table 4: Shapiro-Wilk test of normality - PSQI change from baseline on each visit

	Shapiro-Wilk test			
	Statistic	**df**	**p-value**	**Distribution**
PSQI (6 MONTHS - 0 MONTHS)	0.940	67	0.003	*not normal distribution*
PSQI (12 MONTHS - 0 MONTHS)	0.963	52	0.108	*normal distribution*
PSQI (18 MONTHS - 0 MONTHS)	0.912	29	0.020	*not normal distribution*
PSQI (24 MONTHS - 0 MONTHS)	0.964	20	0.631	*normal distribution*
PSQI (30 MONTHS - 0 MONTHS)	0.845	12	0.032	*not normal distribution*
PSQI (36 MONTHS - 0 MONTHS)	0.801	9	0.021	*not normal distribution*

6.2.3. Missing Data and Accepted Visit Window

All missing data are accounted for each analysis. No imputation of missing data was performed.

All data were organized and analyzed according to the scheduled visits outlined in the approved protocol. Accepted visit window was set to ± 30 days.

6.3. Interim Analyses

Periodic interim data analyses should be performed every 12 months since the start of the study. The last interim analysis was performed at November 2015.

6.4. Determination of Sample Size

Assuming that the standard deviation of the PSQI score would be around 4 (value of the standard deviation 4.04 is taken from Shochat et al. (2007) and not taking into account correlation between PSQI values at times T1 and T2, to detect the mean difference of 1.5 points between global PSQI score at time T1 and T2 with power 0.9 and significance level 0.05 we will need 151 observations when using paired t-test. In reality, a positive correlation between PSQI scores at times T1 and T2 is expected, so that the real power will be higher. In other words, this means that even smaller mean difference will be detectable with power 0.9.

In the case of non-normality of the PSQI scores and the use of the Wilcoxon signed-rank test, 175 observations are needed in the worst case, to obtain the same properties of the test specified above.

It was planned that approximately 200 subjects would be enrolled within 2 years in 10 neurology sites in Slovakia specialized in MS diagnosis, therapy and long-term follow-up observation. Since it turned out, that enrolment of patient was slower than expected; the recruitment had been open also for patients from Hungary and Poland during the year 2014.

7. STUDY SUBJECTS

The study projection was for 200 subjects to participate in the study; 137 subjects were screened and 96 were successfully enrolled and analyzed (although end of study visit was available in 91 patients as 5 withdrawn patients had unavailable last page of CRF).

7.1. Subject Accountability

The international, multicenter study SLK-TYS-11-10230 (LATYSS) was carried out in 3 countries: Slovakia, Poland, and Hungary. The overview of enrolled patients in total and in each country is provided in Table 5.

Table 5: Subjects Population – Screened Subjects

	Total	Slovakia	Hungary	Poland
Number of Screened patients	137	94	19	24
Number of Screen Failures	41	28	3	10
Number of Enrolled and treated patients	96	66	16	14
Number of Completed patients	11	11	0	0
Number of Discontinued patients	85	55	16	14

The first patient in study (02-001) was enrolled in Slovakia on 3rd April 2012 and the last patient (15-001) was enrolled in Hungary on 23rd November 2015. Last visit in study was recorded after the study termination by sponsor on 30th December 2015 (13-001).

Table 6: Number of subjects per site

		N	%
Site number	SK01	12	13%
	SK02	29	30%
	SK03	13	14%
	SK04	6	6%
	SK05	6	6%
	HU10	2	2%
	HU11	4	4%
	HU13	1	1%
	HU14	7	7%
	HU15	1	1%
	HU16	1	1%
	PL20	3	3%
	PL21	3	3%
	PL22	4	4%
	PL24	2	2%
	PL31	2	2%
	Total	**96**	**100%**

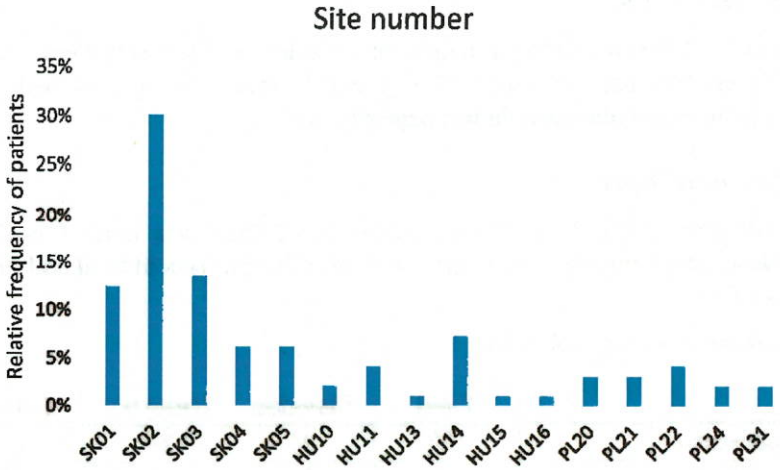

Figure 1: Bar chart of number of subjects per site

The statistical analysis is based on data from all available CRF's from Slovakia, Hungary and Poland. The total count of analyzed patients is 96 although end of study visit is available in 91 patients (5 withdrawn patients have unavailable last page of CRF).

Table 7: Subjects disposition

	Valid visits		Missing visits	
	N	%	N	%
Baseline (0 months)	96	100%	0	0%
6 months	67	70%	29	30%
12 months	52	54%	44	46%
18 months	32	33%	64	67%
24 months	20	21%	76	79%
27 months	20	21%	76	79%
30 months	13	14%	83	86%
33 months	9	9%	87	91%
36 months	9	9%	87	91%
End of Study	91	95%	5	5%

Table 8: Reason for withdrawal

Table 8 summarizes reasons for subject withdrawal. As the sponsor prematurely terminated the study on 27th November 2015 due to insufficient enrollment rate, study termination was the most frequent reason for premature withdrawal.

Table 8: Reason for withdrawal

		N	%
1) IC withdrawal		10	12%
2) Death of patient		0	0%
3) Lost of contact		2	2%
4) Other		68	80%
	a) Study termination	58	69%
	b) Lack of efficacy	4	5%
	c) Treatment switch	3	4%
	d) Allergic reaction to Tysabri	2	2%
	e) Fulminant hepatitis	1	1%
5) Missing		5	6%

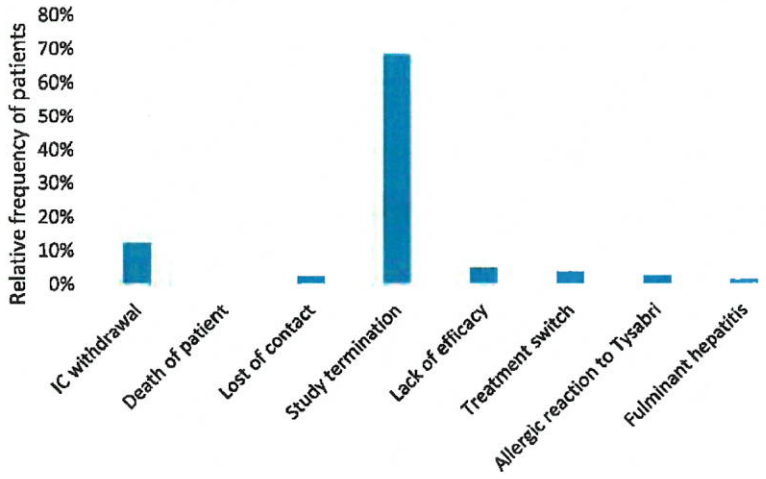

Figure 2: Bar chart of reasons for withdrawal (n = 80)

7.2. Demographics

7.2.1. Distribution of Gender in Patient Population

Among patients with MS, female to male ratio was 2.3 in year 2000 (Alonso and Hernan, 2008) what is in accordance with gender distribution in the study population as shown in Table 9 and Figure 3: Pie chart of genderFigure 3.

Table 9: Gender distribution

	N	%
Male	24	25%
Female	72	75%
Total	**96**	**100%**

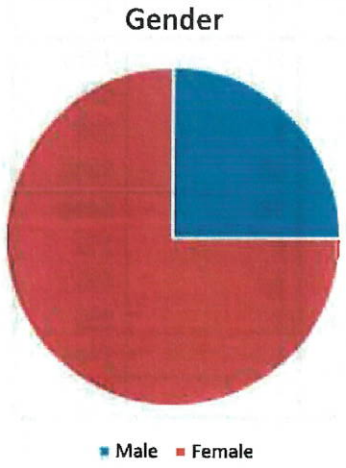

Figure 3: Pie chart of gender

7.2.2. Age Distribution in Patient Population

Table 10: Descriptive statistics of ageTable 10 and Figure 4 present results of descriptive statistics of patients' age at baseline.

Table 10: Descriptive statistics of age

	N	Missing	Mean	Standard Deviation	Median	Minimum	Maximum
Age	96	0	36.4	8.82	36.0	18	57

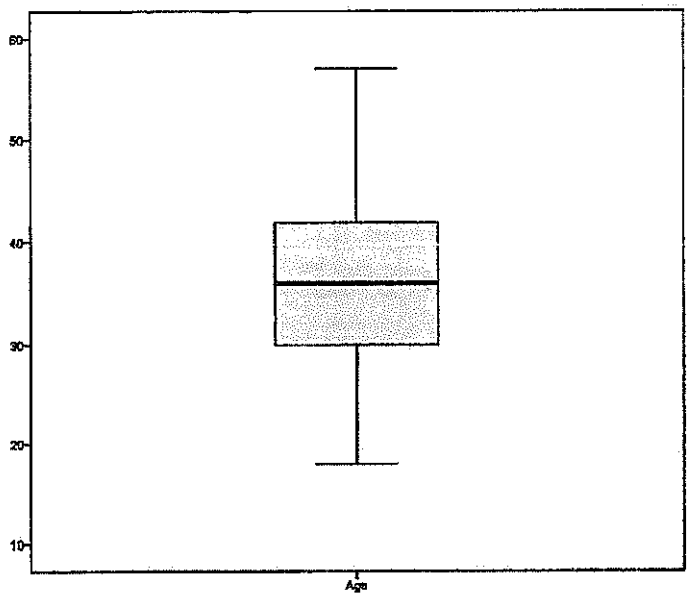

Figure 4: Boxplot of median of age (N = 96)

7.3. Baseline Disease Characteristics

Duration of clinically definite multiple sclerosis (CDMS) diagnosis in baseline was analysed. Mean of the duration is 5.31 years (SD = 4.356 years). Time from baseline to the last attack is measured in months, the mean value is 7.47 months (SD = 14.252 months). The distribution of these times is presented in Table 11 and following graphs.

Table 11: Descriptive statistics of baseline disease characteristics

	N	Missing	Mean	SD	Median	Minimum	Maximum
CDMS [years]	96	0	5.31	4.356	4.5	0	21
Last attack before study [months]	49	47	7.47	14.252	4	0	86

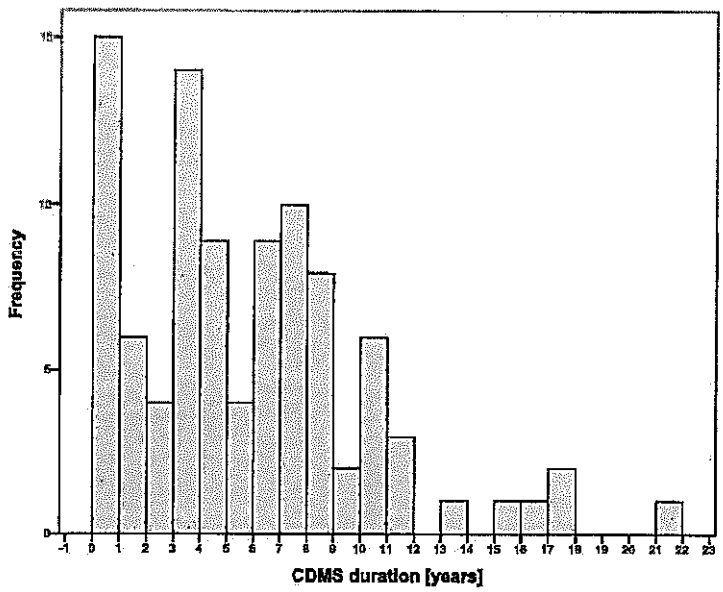

Figure 5: Histogram of CDMS duration at baseline (N = 96)

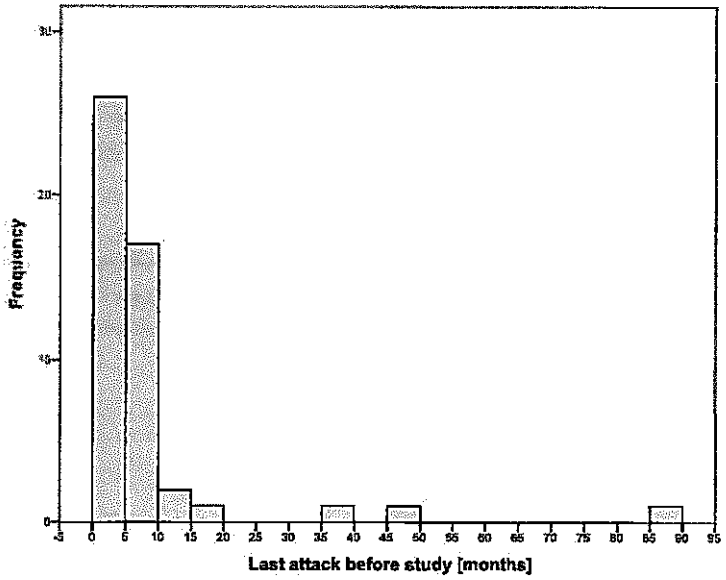

Figure 6: Histogram of last MS attacks at baseline (N = 49)

8. STUDY RESULTS

8.1. Primary endpoint – PSQI

The PSQI has range of total score from 0 to 21. In scoring the PSQI, seven component scores are derived. Higher scores indicate worse sleep quality. Total score less or equal to 5 is associated with good sleep quality and total score greater than 5 is associated with poor sleep quality.

8.1.1. Descriptive Statistics

Table 12 describes the number of collected PSQI questionnaires during the study visits, while the following tables and graphs show descriptive statistics of PSQI itself or PSQI change from baseline to each visit.

Table 12: Number of collected PSQI questionnaires

	yes		no	
	N	%	N	%
PSQI collected (0 MONTHS)	96	100%	0	0%
PSQI collected (6 MONTHS)	67	100%	0	0%
PSQI collected (12 MONTHS)	52	100%	0	0%
PSQI collected (18 MONTHS)	29	91%	3	9%
PSQI collected (24 MONTHS)	20	100%	0	0%
PSQI collected (30 MONTHS)	12	92%	1	8%
PSQI collected (36 MONTHS)	9	100%	0	0%

Table 13: Descriptive statistics of PSQI

PSQI	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	96	7.4	3.14	6.0	5	18
6 MONTHS	67	6.1	3.51	6.0	1	17
12 MONTHS	52	7.1	3.56	6.0	1	17
18 MONTHS	29	6.7	3.00	6.0	3	15
24 MONTHS	20	4.8	2.42	4.5	1	11
30 MONTHS	12	5.3	2.71	5.0	2	12
36 MONTHS	9	6.4	3.57	6.0	2	12

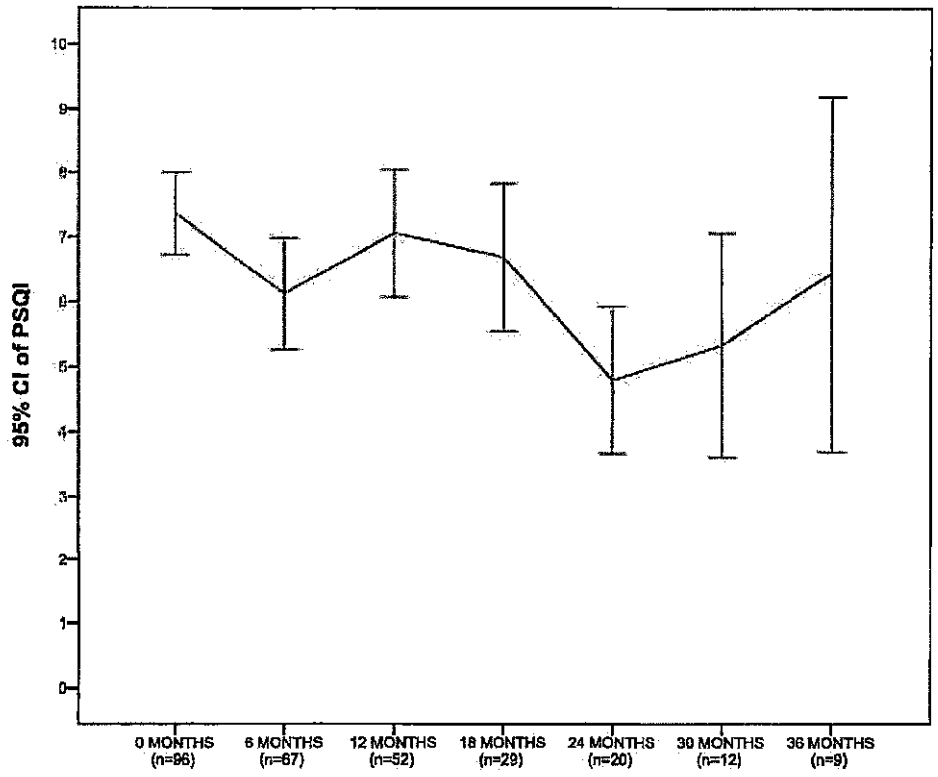

Figure 7: Line graph of mean of PSQI during the study

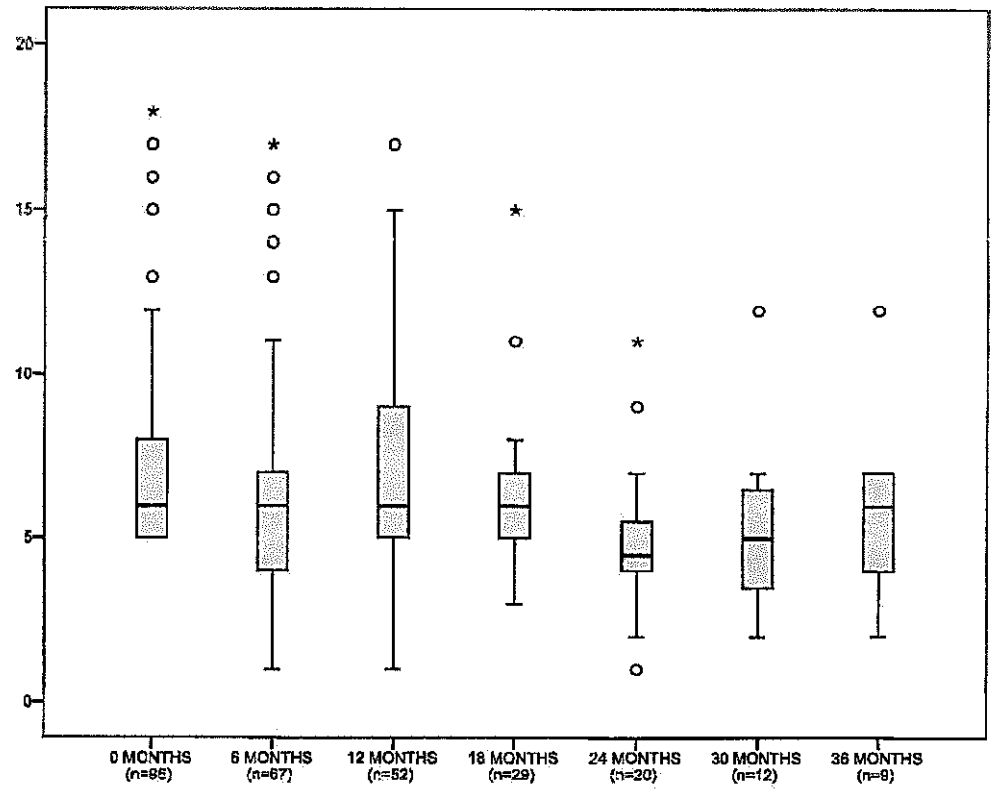

Figure 8: Boxplot of median of PSQI during the study

Table 14: Descriptive statistics of PSQI change from baseline on each visit

PSQI	N	Mean	Standard Deviation	Median	Minimum	Maximum	95% Confidence interval for Mean*	
6 - 0 MONTHS	67	-1.1	2.89	0.0	-11	6	-1.76	-.36
12 - 0 MONTHS	52	-0.4	2.74	0.0	-8	6	-1.13	0.40
18 - 0 MONTHS	29	-0.8	2.34	0.0	-6	6	-1.65	0.13
24 - 0 MONTHS	20	-2.1	2.87	-1.5	-8	4	-3.40	-0.70
30 - 0 MONTHS	12	-1.7	2.53	-1.0	-7	1	-3.28	-0.06
36 - 0 MONTHS	9	-0.3	3.20	-1.0	-3	7	-2.79	2.13

*Mean Rank is used for Friedman test

Table 15: Descriptive statistics and ranks for test for several related samples of PSQI (0 - 12 MONTHS)

PSQI	N	Mean	Standard Deviation	Median	Minimum	Maximum	95% Confidence interval for Mean		Mean Rank*
0 MONTHS	47	7.5	3.30	6.0	5	18	6.52	8.46	2.2
6 MONTHS	47	6.7	3.63	6.0	1	17	5.64	7.77	1.8
12 MONTHS	47	7.1	3.56	6.0	1	17	6.04	8.13	2.0

*Mean Rank is used for Friedman test

Table 16: Descriptive statistics and ranks for test for several related samples of PSQI (0 - 6 MONTHS)

PSQI	N	Mean	Standard Deviation	Median	Minimum	Maximum	95% Confidence interval for Mean		Mean Rank*
0 MONTHS	67	7.2	2.98	6.0	5	18	6.45	7.91	1.6
6 MONTHS	67	6.1	3.51	6.0	1	17	5.26	6.98	1.4

*Mean Rank is used for Friedman test

8.1.2. Hypotheses Testing

Hypotheses described in detail above (section 6.2.1) were tested with stated methods (Table 17-Table 19). As a result, significant PSQI change from baseline was confirmed between visit in Month 6 (P = 0,004, N=67); however, differences in PSQI among baseline, Month 6 and Month 12 were not significant. The statistically significant change in Month 6 was negative indicating that, sleep quality was improving. No correlation between PSQI at any time point and number of T2 lesions was confirmed on the level of significance 0.05. Due to low number of cases (< 30), the tests were omitted at visits Month 18, 24, 30 and 36 as the results could be irrelevant.

Table 17: Nonparametric one-sample Wilcoxon signed rank test – PSQI change from baseline on visits Month 6 and 12

	N	W	Z	p-value	Conclusion
PSQI (6 MONTHS – 0 MONTHS)	67	296.5	-2.847	0.004	*H1 is confirmed*
PSQI (12 MONTHS – 0 MONTHS)	52	261.5	-0.881	0.378	*H2 is not confirmed*

Table 18: Nonparametric Friedman test for several related samples - PSQI on visits Month 0, 6 and 12

	N	Chi-Square	df	p-value	Conclusion
PSQI on each visit (Month 0, 6, 12)	47	4.168	2	0.124	*H11 is not confirmed*
PSQI on each visit (Month 0, 6)	67	7.681	1	0.006	*H12 is confirmed*

Table 19: Spearman correlation coefficient - T2 lesions and PSQI on visit Month 0 and 12

	Number of T2 lesions			Conclusion
	N	Correlation Coefficient	p-value	
PSQI (0 MONTHS)	90	-0.092	0.386	*H13 is not confirmed*
PSQI (12 MONTHS)	49	0.115	0.433	*H14 is not confirmed*

8.2. Secondary endpoints

8.2.1. Impact of the Tysabri® Treatment on Fatigue – MFIS

The full-length MFIS consists of 21 items while the abbreviated version has 5 items. The Total MFIS score can range from 0-84 (0 is the least and 84 is the greatest fatigue). It is computed by adding scores on the Physical, Cognitive, and Psychosocial subscales. The physical subscale can range from 0-36, the cognitive subscale can range from 0-40 and the psychosocial subscale can range from 0-8.

Table 20: Number of collected MFIS questionnaires

	yes		no	
	N	%	N	%
MFIS collected (0 MONTHS)	95	99%	1	1%
MFIS collected (6 MONTHS)	67	100%	0	0%
MFIS collected (12 MONTHS)	52	100%	0	0%
MFIS collected (18 MONTHS)	29	91%	3	9%
MFIS collected (24 MONTHS)	20	100%	0	0%
MFIS collected (30 MONTHS)	13	100%	0	0%
MFIS collected (36 MONTHS)	9	100%	0	0%

As summarized in

Table 21 and on Figure 9 and Figure 10, MFIS shows slight improvement during the study, although data from month 30 and 36 had higher variability most probably due to lower number of evaluated patients.

Table 21: Modified Fatigue Impact Scale

MFIS	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	95	36.6	17.88	38.0	2	74
6 MONTHS	67	32.7	17.58	34.0	0	67
12 MONTHS	52	31.3	17.90	32.0	0	66
18 MONTHS	29	33.7	17.04	33.0	2	66
24 MONTHS	20	30.5	15.53	31.5	1	53
30 MONTHS	13	36.1	18.40	40.0	3	73
36 MONTHS	9	27.7	18.15	33.0	3	51

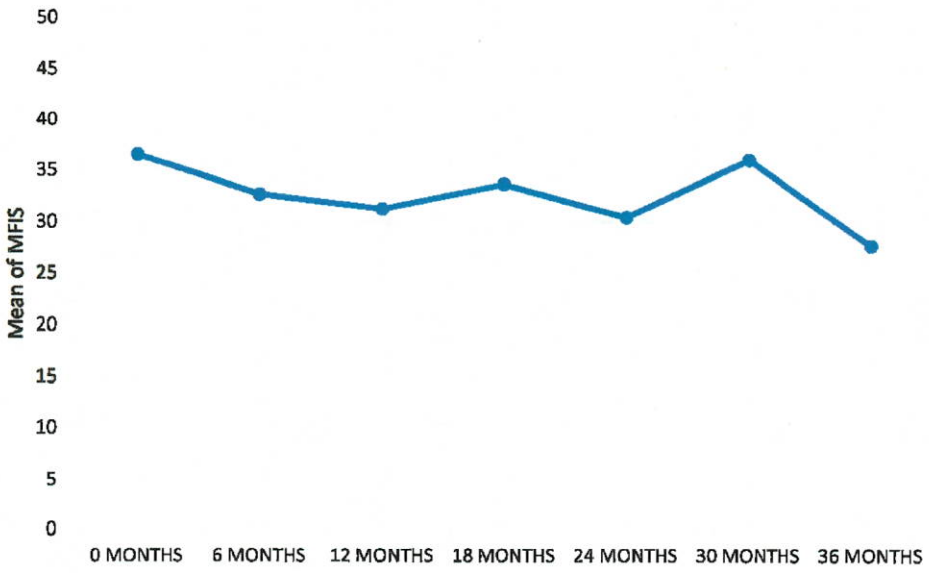

Figure 9: Line graph of mean of MFIS score during the study

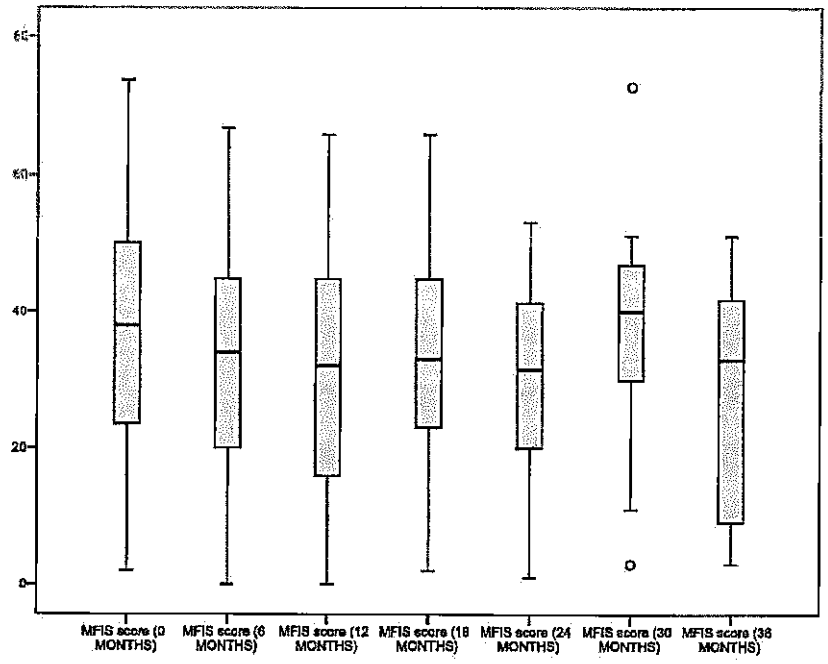

Figure 10: Boxplot of median of MFIS score during the study

8.2.2. Impact of the Tysabri® treatment on daily sleepiness – ESS

The ESS asks people to rate, on a 4-point scale (0 – 3), their usual chances of dozing off or falling asleep in 8 different situations or activities that most people engage in as part of their daily lives, although not necessarily every day. The total ESS score is the sum of 8 item-scores and can range between 0 and 24. The higher is the score, the higher is the person's level of daytime sleepiness.

Table 22: Number of collected ESS questionnaires

	yes		no	
	N	%	N	%
ESS collected (0 MONTHS)	95	99%	1	1%
ESS collected (6 MONTHS)	67	100%	0	0%
ESS collected (12 MONTHS)	51	98%	1	2%
ESS collected (18 MONTHS)	29	91%	3	9%
ESS collected (24 MONTHS)	20	100%	0	0%
ESS collected (30 MONTHS)	13	100%	0	0%
ESS collected (36 MONTHS)	8	89%	1	11%

As described in Table 23 and on Figure 11 and Figure 12, ESS shows improvement during the study, although especially data from month 30 and 36 were collected from lower number of patients compared to the other time points.

Table 23: Ephworth Sleepiness Scale

ESS	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	95	6.3	4.29	6.0	0	19
6 MONTHS	67	6.4	4.85	6.0	0	24
12 MONTHS	51	6.0	4.31	5.0	0	19
18 MONTHS	29	5.1	3.33	4.0	0	14
24 MONTHS	20	4.8	3.44	4.5	0	12
30 MONTHS	13	6.2	4.22	7.0	0	14
36 MONTHS	8	4.1	4.22	3.5	0	10

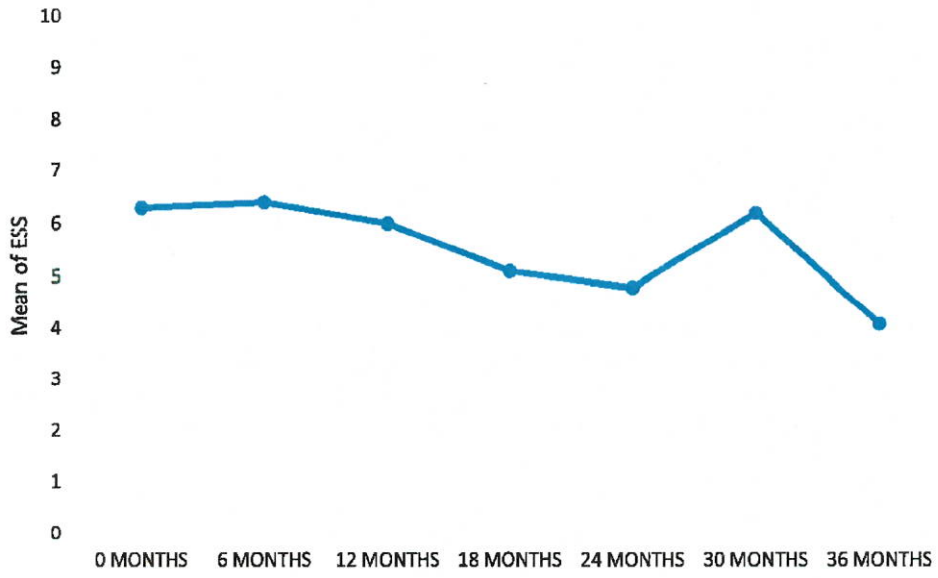

Figure 11: Line graph of mean of ESS score during the study

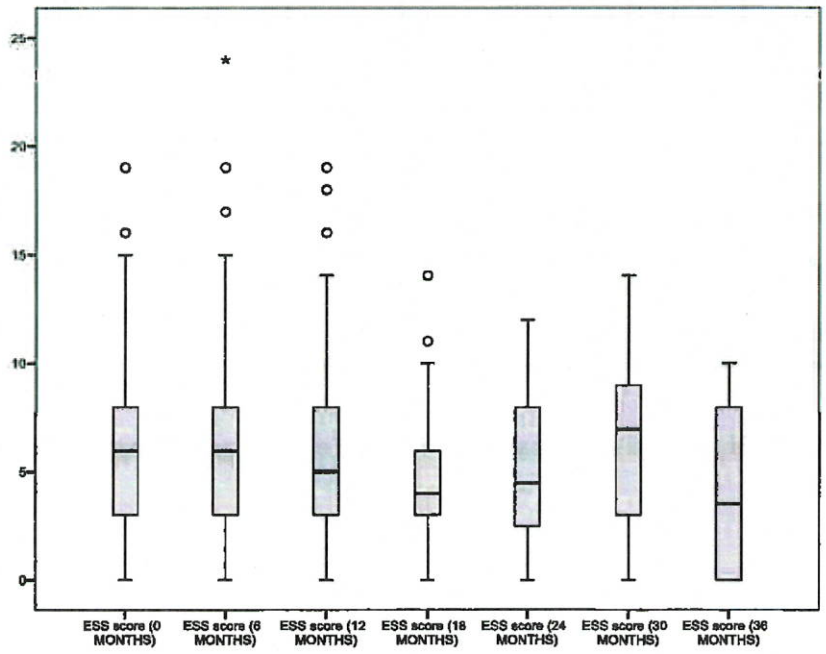

Figure 12: Boxplot of median of ESS score during the study

8.2.3. Impact of the Tysabri® treatment on subjects' quality of life – EQ-5D

The EQ-5D-3L essentially consists of 2 pages - the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D-3L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, extreme problems. The result from each dimension in a 1-digit number (from 1-3) express the level selected for that dimension. The digits for 5 dimensions can be combined in a 5-digit number describing the respondent's health state. The profile 33333 indicates the worse and the profile 11111 indicates the best quality of life.

These profiles represent 243 theoretically possible health states defined by EQ-5D (list of value sets). Value set is set of national population-based utility weights to evaluation of quality of life. Since, there is absence of a set of national population-based utility weights in Slovakia, Hungary and Poland then the UK value set of utility weights is selected for a study population. The final value can range from 0 to 1, when 0 is the worst and 1 is the best quality of life.

Table 24: Number of collected EQ-5D questionnaires

	yes		no	
	N	%	N	%
EQ-5D (0 MONTHS)	95	99%	1	1%
EQ-5D (6 MONTHS)	67	100%	0	0%
EQ-5D (12 MONTHS)	52	100%	0	0%
EQ-5D (18 MONTHS)	28	88%	4	13%
EQ-5D (24 MONTHS)	20	100%	0	0%
EQ-5D (30 MONTHS)	13	100%	0	0%
EQ-5D (36 MONTHS)	9	100%	0	0%

Both EQ-VAS and EQ-5D-3L do not show any trend during the study as summarized in the following tables and figures.

Table 25: EQ VAS

EQ VAS	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	94	64.9	18.41	66.5	20	100
6 MONTHS	66	68.5	18.42	71.5	15	99
12 MONTHS	52	70.3	18.18	75.0	15	100
18 MONTHS	28	69.3	15.90	70.0	26	100
24 MONTHS	20	68.4	16.48	69.5	32	95
30 MONTHS	13	70.2	20.42	75.0	28	100
36 MONTHS	9	63.2	25.49	75.0	25	100

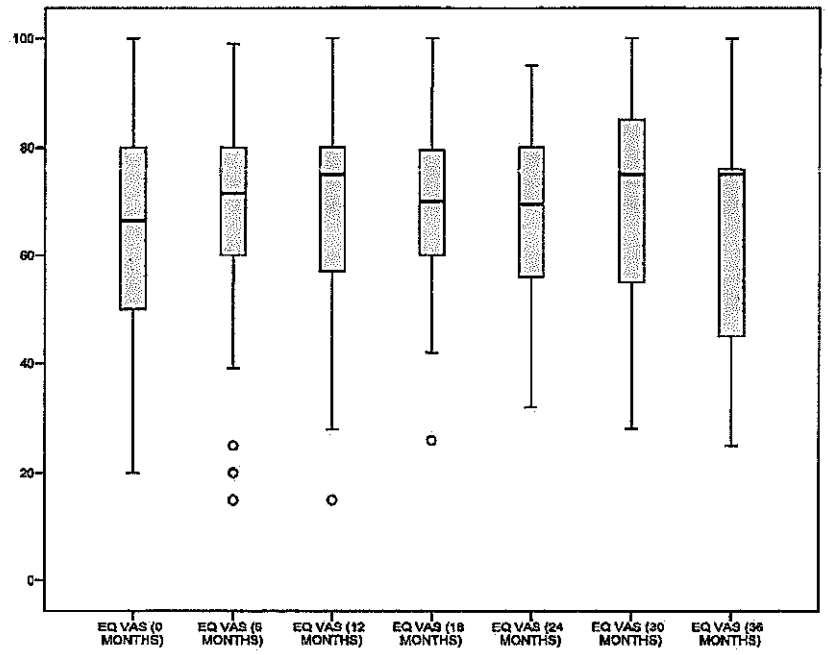

Figure 13: Boxplot of median of EQ VAS value during the study

Table 26: EQ-5D-3L

EQ-5D-3L	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	93	0.768	0.1219	0.739	0.57	1.00
6 MONTHS	66	0.801	0.1265	0.816	0.59	1.00
12 MONTHS	50	0.786	0.1410	0.735	0.59	1.00
18 MONTHS	28	0.779	0.1264	0.743	0.59	1.00
24 MONTHS	20	0.832	0.1452	0.807	0.59	1.00
30 MONTHS	13	0.776	0.1319	0.740	0.59	1.00
36 MONTHS	9	0.753	0.1236	0.679	0.65	1.00

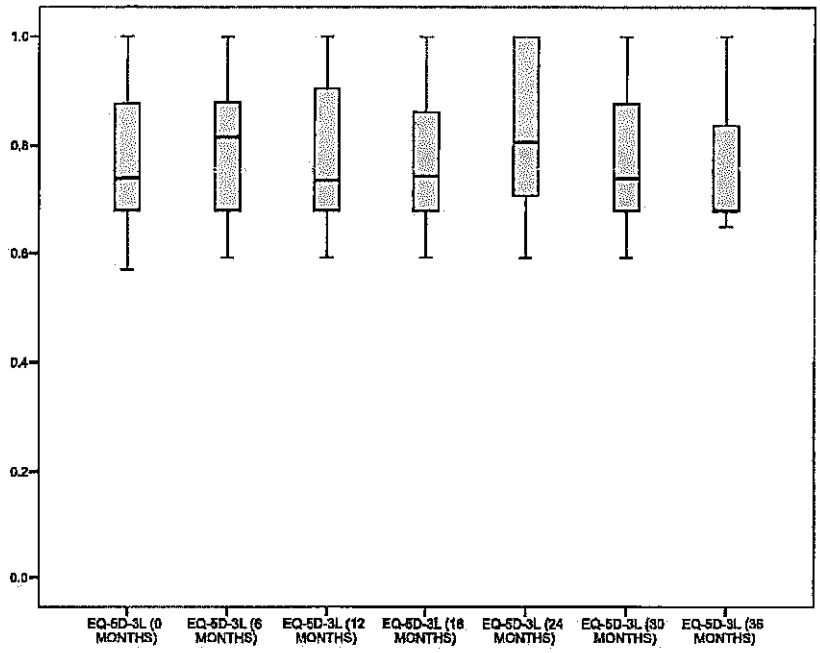

Figure 14: Boxplot of median of EQ-5D-3L value during the study

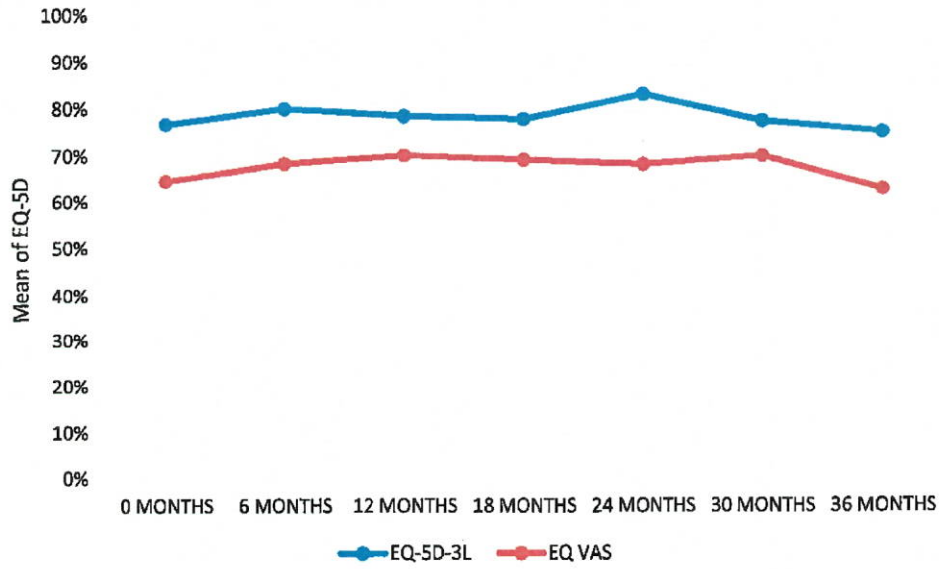

Figure 15: Line graph of mean of EQ-5D-3L and EQ-VAS score during the study

8.2.4. Assessment of existence and severity of symptoms of depression – BDI-II

The BDI-II questionnaire consists of 21 groups of statements. Each question can range from 0-3. Total score is between 0 and 63, when 63 is the worst and 0 is the best result (no depression).

Table 27: Number of collected BDI-II questionnaires

	yes		no	
	N	%	N	%
BDI-II (0 MONTHS)	95	99%	1	1%
BDI-II (6 MONTHS)	67	100%	0	0%
BDI-II (12 MONTHS)	52	100%	0	0%
BDI-II (18 MONTHS)	29	91%	3	9%
BDI-II (24 MONTHS)	20	100%	0	0%
BDI-II (30 MONTHS)	13	100%	0	0%
BDI-II (36 MONTHS)	9	100%	0	0%

Different versions of BDI-II questionnaire were collected in Hungary and Poland (standardized questionnaire) and in Slovakia (modified version). Hence, the assessment of this two different versions BDI-II questionnaire is made separately.

However, the improvement of BDI-II could be seen in both versions as it is described in Table 28, Table 29 and the following figures. For months 30 and 36 in the Slovakian population, the increase of BDI-II could be probably explained by the higher variability of data caused by small number of assessed patients.

8.2.4.1. Results from Hungary and Poland

Table 28: BDI-II in Hungary and Poland

BDI-II	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	30	13.1	7.52	13.0	0	32
6 MONTHS	17	11.1	8.40	11.0	1	29
12 MONTHS	14	10.8	8.07	7.5	0	24
18 MONTHS*	1*	8	-	8	8	8
24 MONTHS	0	-	-	-	-	-
30 MONTHS	0	-	-	-	-	-
36 MONTHS	0	-	-	-	-	-

*BDI-II total score (18 MONTHS) is constant. It has been omitted in boxplot.

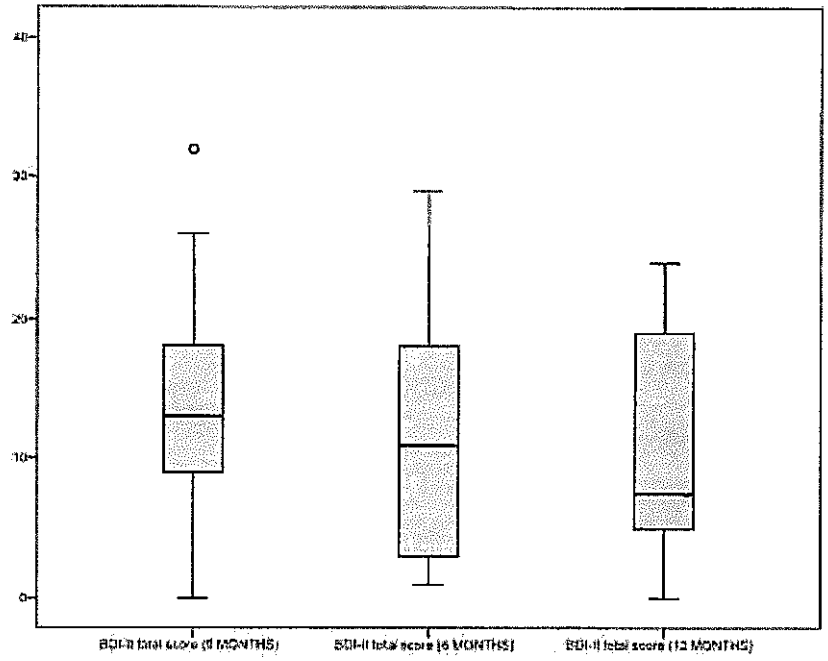

Figure 16: Boxplot of median of BDI-II score in Hungary and Poland during the study

8.2.4.2. Results from Slovakia

Table 29: BDI-II in Slovakia

BDI-II	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	65	10.9	7.91	9.0	0	35
6 MONTHS	50	8.9	7.47	7.0	0	33
12 MONTHS	38	8.7	6.28	7.0	0	25
18 MONTHS	28	7.5	5.89	6.0	0	22
24 MONTHS	20	6.6	6.12	4.0	0	19
30 MONTHS	13	9.3	8.04	12.0	0	21
36 MONTHS	9	11.0	10.84	10.0	0	33

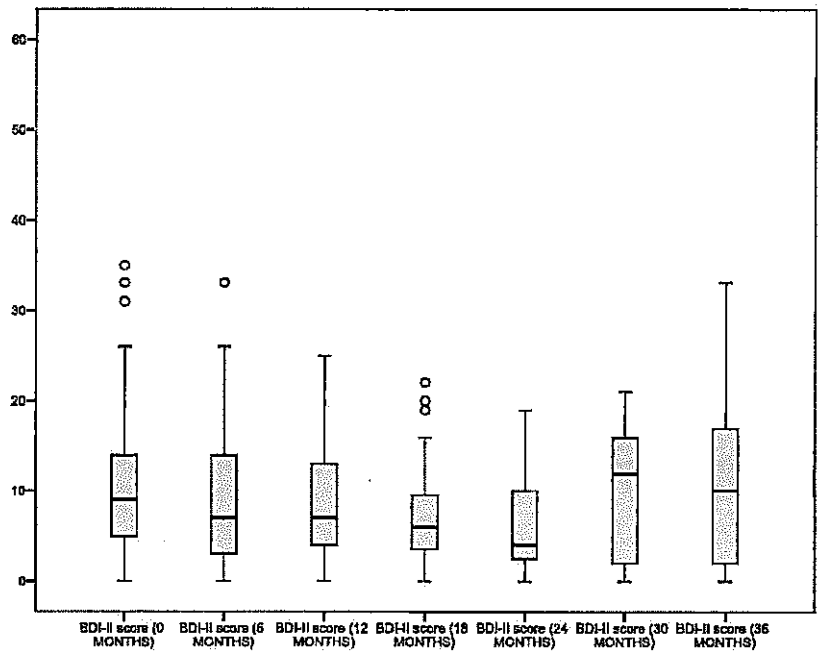

Figure 17: Boxplot of median of BDI-II score in Slovakia during the study

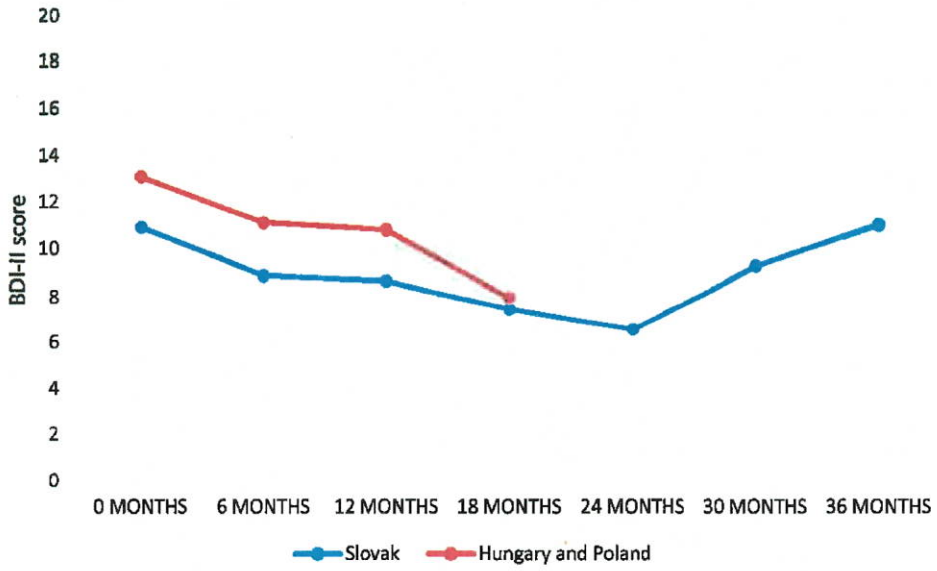

Figure 18: Line graph of mean of BDI-II score during the study

8.2.5. Occurrence of Clinical Relapses – ARR

After the first 6 months of treatment in the study, the percentage of patients without MS attack in previous 6 months rose from 14.0% at baseline to 95 - 100% at the other assessed time points. Similarly, the percentage of patients with one MS attack in previous 6 months dropped from 57% at baseline to 0 - 6% at the other assessed time points. Furthermore, the percentage of patients with two MS attacks in previous 6 months dropped from 26% at baseline to 0% at the other assessed time points. At last, there were 3 patients with 3 MS attacks in previous 6 months at baseline, but no case of 3 MS attacks in previous 6 months was reported during the study.

Table 30: Number of subjects with relapses

Number of relapses (visit)	0		1		2		3		missing
	N	%	N	%	N	%	N	%	N
Within last 6 months (Month 0)	13	14%	53	57%	24	26%	3	3%	3
Since previous visit (Month 6)	66	99%	1	1%	0	0%	0	0%	0
Since previous visit (Month 12)	50	100%	0	0%	0	0%	0	0%	2
Since previous visit (Month 18)	23	96%	1	4%	0	0%	0	0%	8
Since previous visit (Month 24)	18	95%	1	5%	0	0%	0	0%	1
Since previous visit (Month 30)	13	100%	0	0%	0	0%	0	0%	0
Since previous visit (Month 36)	9	100%	0	0%	0	0%	0	0%	0

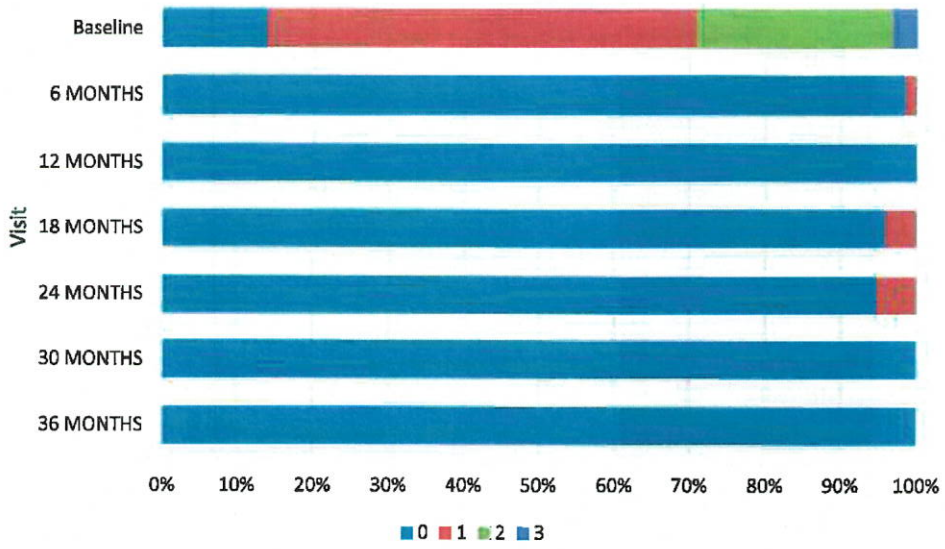

Figure 19: Bar chart of relapses during the study

ARR is defined as the total number of confirmed relapses divided by the number of days of observation x 365.25 for each subject. Mean of ARR was improved during the study (from 2.37 to 0.03 relapses per year).

Table 31: Occurrence of ARR

ARR	N	Mean	Standard Deviation	Median	Minimum	Maximum
prior the study	93	2.37	1.413	2.0	0.0	6.0
during the study	80	0.03	0.133	0.0	0.0	0.9

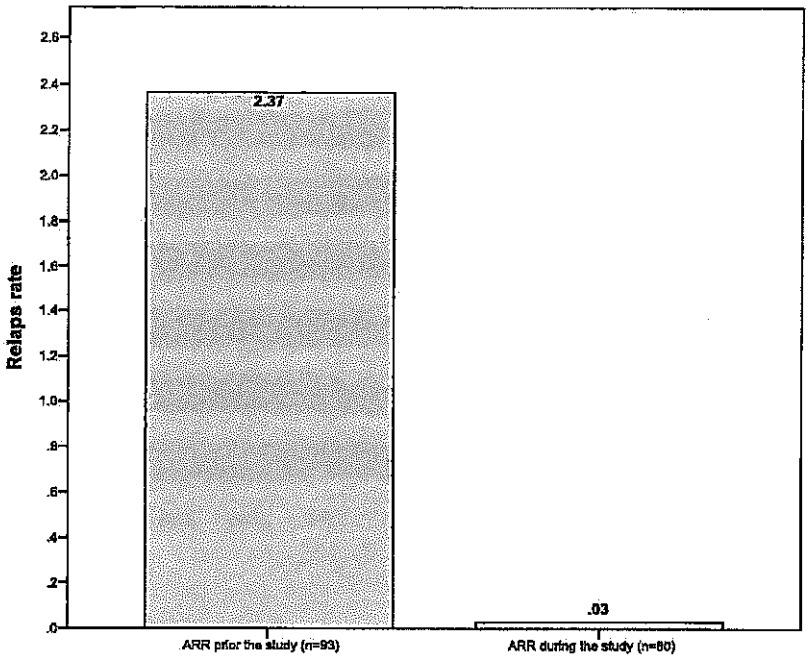

Figure 20: Bar chart of ARR before and during the study

8.2.6. Assessment of Disability Progression Evaluated by Physician – EDSS

EDSS provides a total score on a scale that ranges from 0 to 10. The first levels 1.0 to 4.5 refer to people with a high degree of ambulatory ability and the subsequent levels 5.0 to 9.5 refer to the loss of ambulatory ability. The range of main categories include (0) = normal neurologic exam; to (5) = ambulatory without aid or rest for 200 meters; disability severe enough to impair full daily activities; to (10) = death due to MS. In addition, it also provides eight subscale measurements called Functional System (FS) scores. These subscale categories are listed below. The levels of function within each category refer to the eight functional systems affected by MS.

EDSS was assessed every 6 months. During the study, the score was varying with slightly increasing trend as described in the

Table 33. The median value remained the same for the whole study (3.0-4.0). The mean values are: 2.92 at baseline, 3.01 at month 6, 3.14 at month 12, 3.48 at month 18, 3.53 at month 24, 3.38 at month 30, and 3.61 at month 36.

Table 32: Number of collected EDSS questionnaires

	yes		**no**	
	N	%	N	%
EDSS (0 MONTHS)	96	100%	0	0%
EDSS (6 MONTHS)	61	91%	6	9%
EDSS (12 MONTHS)	52	100%	0	0%
EDSS (18 MONTHS)	29	91%	3	9%
EDSS (24 MONTHS)	19	95%	1	5%
EDSS (30 MONTHS)	13	100%	0	0%
EDSS (36 MONTHS)	9	100%	0	0%

Table 33: EDSS assessed every 6 months

	N	Mean	Standard Deviation	Median	Minimum	Maximum
EDSS (0M)	96	2.92	0.955	3.00	0.0	4.0
EDSS (6M)	61	3.01	1.043	3.00	0.0	4.5
EDSS (12M)	52	3.14	1.169	3.00	0.0	6.5
EDSS (18M)	29	3.48	0.930	4.00	1.0	5.0
EDSS (24M)	19	3.53	0.634	3.50	2.0	4.5
EDSS (30M)	13	3.38	1.003	4.00	1.0	4.5
EDSS (36M)	9	3.61	1.167	4.00	1.0	5.0

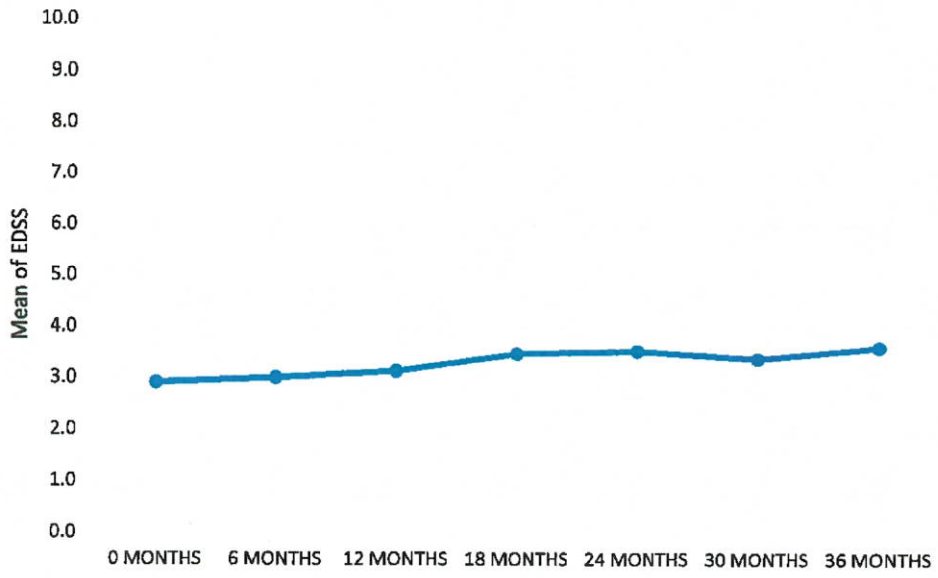

Figure 21: Line graph of mean of EDSS score during the study

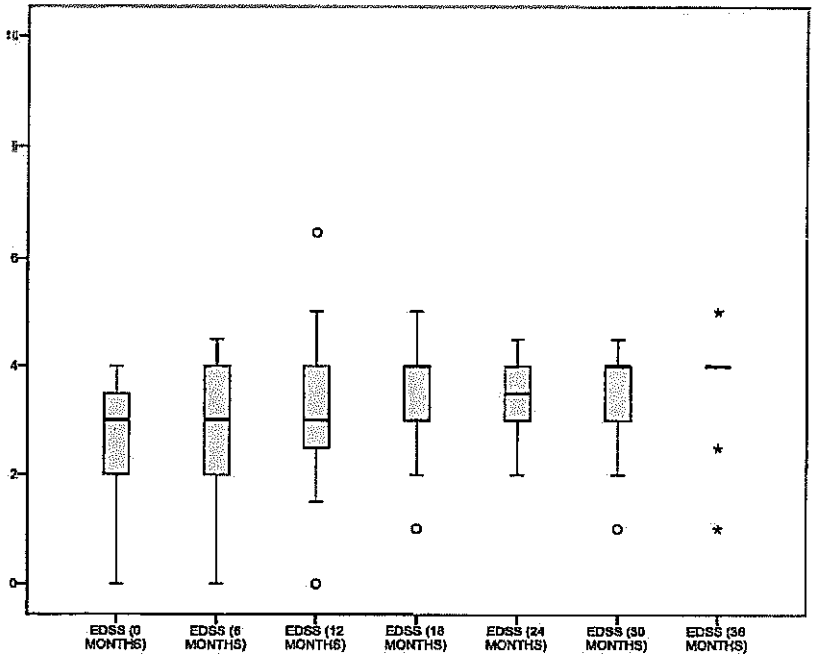

Figure 22: Boxplot of median of EDSS value during the study

8.2.7. Assessment Cognitive Functions – MoCA

MoCA was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The total possible score is 30 points; a score of 26 or above is considered normal. Total score is sum of all subscores listed on the right-hand side. One point has to be added for an individual who has 12 years or fewer of formal education, for a possible maximum of 30 points. A final total score of 26 and above is considered normal.

According to the protocol, MoCA test was not collected at month 6, but some investigators put forward a test to patients in this visit (see

Table 34).

In the study, there were used different versions of MoCA test: 7.1, 7.2, and 7.3. All versions are compatible; therefore, all versions are analyzed together.

No trend in MoCA could be found as shown in Table 35 and Figure 23 and Figure 24.

Table 34: Number of collected MoCA questionnaires

	yes		no	
	N	%	N	%
MOCA collected (0 MONTHS)	95	99%	1	1%
MOCA collected (6 MONTHS)	3	4%	64	96%
MOCA collected (12 MONTHS)	50	96%	2	4%
MOCA collected (18 MONTHS)	8	25%	24	75%
MOCA collected (24 MONTHS)	19	95%	1	5%
MOCA collected (27 MONTHS)	20	100%	0	0%
MOCA collected (30 MONTHS)	0	0%	13	100%
MOCA collected (33 MONTHS)	9	100%	0	0%
MOCA collected (36 MONTHS)	7	78%	2	22%

Table 35: MoCA assessment

	N	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	95	26.8	3.18	28.0	13	30
6 MONTHS	3	24.0	1.00	24.0	23	25
12 MONTHS	50	27.2	2.67	28.0	19	30
18 MONTHS	8	27.1	1.64	27.0	24	29
24 MONTHS	19	26.9	2.68	27.0	21	30
27 MONTHS	20	26.8	3.71	28.0	18	30
30 MONTHS	0*	-	-	-	-	-
33 MONTHS	9	27.3	2.78	28.0	21	30
36 MONTHS	7	27.3	3.35	28.0	20	30

*The MoCA was not collected. It has been omitted.

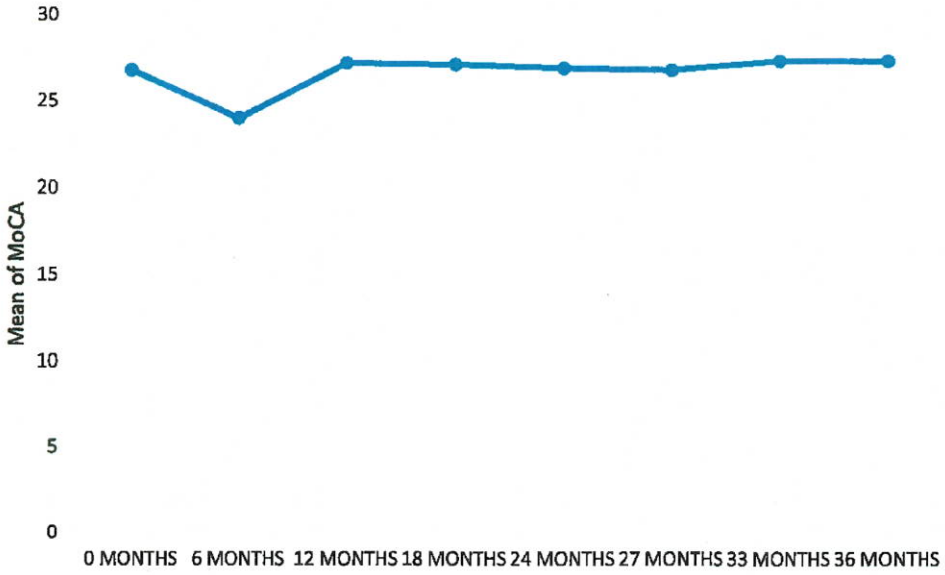

Figure 23: Line chart of mean of MoCA during the study

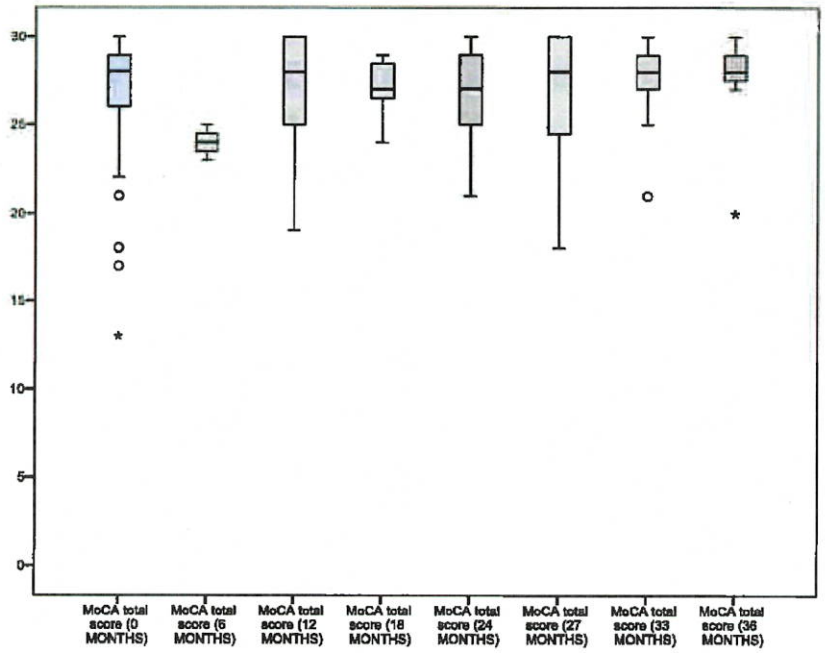

Figure 24: Boxplot of median of MoCA test during the study

8.2.8. Impact of the Disease on Subject's Employment

A comparison between the number of employed patients at the baseline and at the end of study was made in pairs, 86 patients had this question complete at both visits. The number of employed or economically active patients slightly decreased during the study (62% at baseline and 51% at end of treatment visit-EOS). There were only mild changes in characteristics of employment during the study: full time/part time (baseline 83%/18% and EOS 81%/19%); manual/intellectual (baseline 27%/73% and EOS 20%/80%) as shown in the following tables and figures.

Table 36: Number of employed or economically active patients

	Employed or economically active (Month 0)		Employed or economically active (EOS)	
	N	%	N	%
yes	53	62%	44	51%
no	33	38%	42	49%

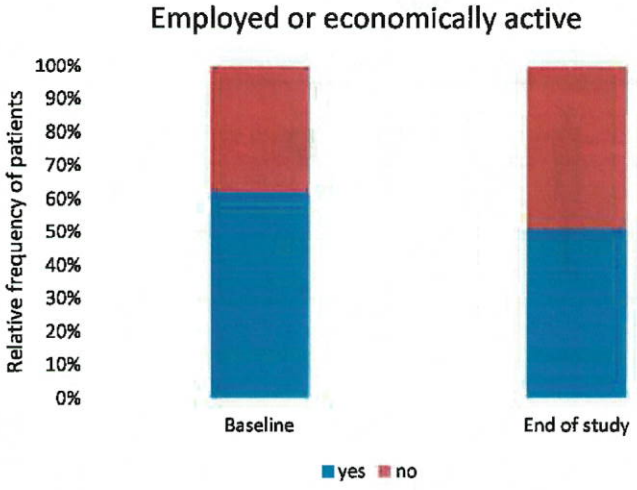

Figure 25: Bar chart of employed or economically active patients

Table 37: Number of full-time / part-time employed patients

	Employment (Month 0)		Employment (EOS)	
	N	%	N	%
part time	7	18%	8	19%
full time	33	83%	35	81%

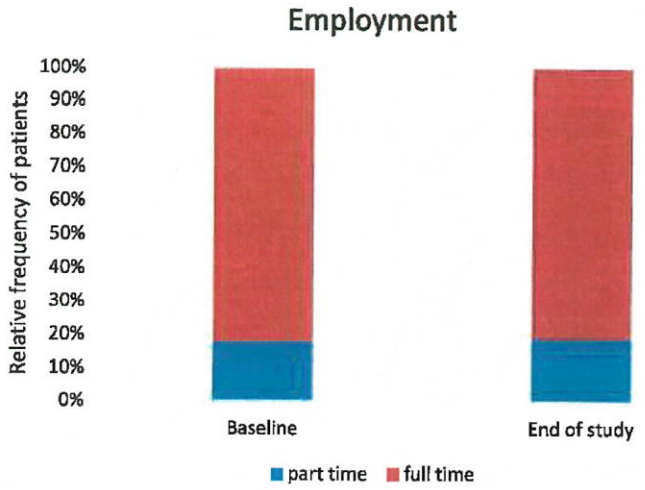

Figure 26: Bar chart of full-time / part-time employed patients

Table 38: Last or current employment of patients

	Last or current employment (Month 0)		Last or current employment (EOS)	
	N	%	N	%
manual	17	27%	10	20%
intellectual	45	73%	39	80%

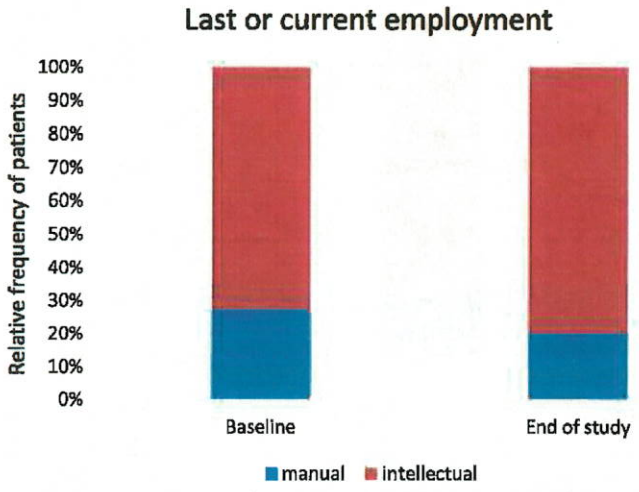

Figure 27: Bar chart of last or current employment of patients

8.2.9. Assessment of MRI Changes – T2 Lesions, Gd-enhancing Lesions

During the study, MRI assessments were performed every 12 months. While number of T2 lesion increased during the study (Table 40: Number of T2-lesionsTable 40), number of active Gd-enhancing lesions clearly dropped to 0 after the first year of Tysabri® treatment (Table 41).

Table 39: Number of performed MRI examinations every year

	yes		no	
	N	%	N	%
MRI (0 MONTHS)	95	99%	1	1%
MRI (12 MONTHS)	51	98%	1	2%
MRI (24 MONTHS)	19	95%	2	5%
MRI (36 MONTHS)	8	89%	1	11%

Table 40: Number of T2-lesions

	N	Missing	Mean	Standard Deviation	Median	Minimum	Maximum
0 MONTHS	90	6	36.6	23.28	31.5	8	126
12 MONTHS	49	47	39.5	21.72	36.0	9	93
24 MONTHS	19	77	43.6	19.14	42.0	17	82
36 MONTHS	7	89	53.0	32.61	50.0	9	100

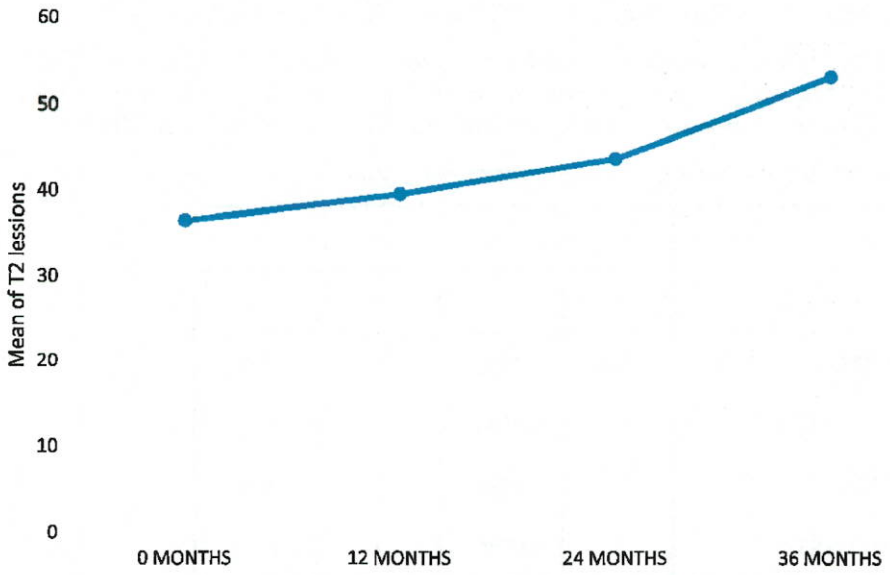

Figure 28: Line graph of mean of T2 lesions during the study

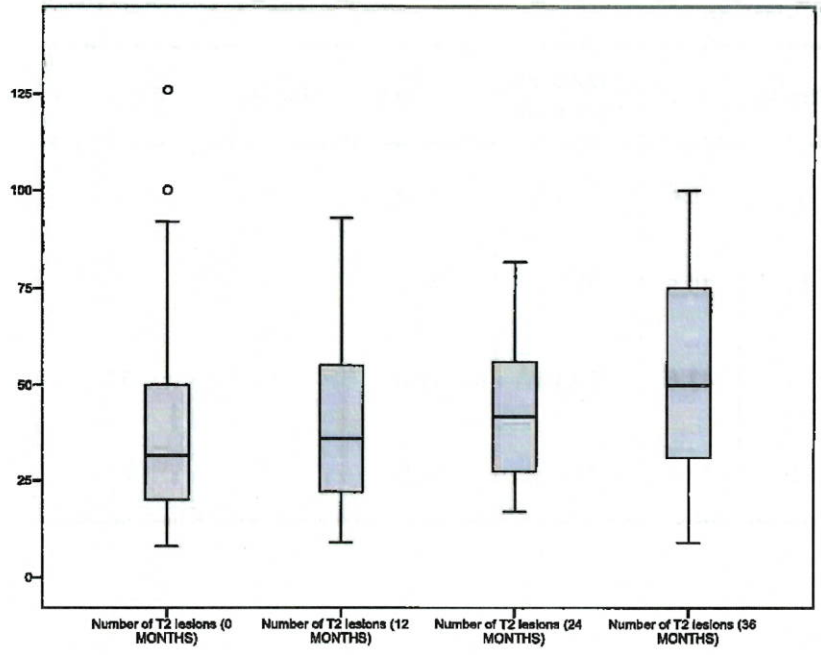

Figure 29: Boxplot of median of number of T2 lesions during the study

Table 41: Number of Gd-enhancing lesions

	0		1		2		3 and more	
	N	%	N	%	N	%	N	%
0 MONTHS	50	60%	10	12%	6	7%	17	20%
12 MONTHS	45	100%	0	0%	0	0%	0	0%
24 MONTHS	15	100%	0	0%	0	0%	0	0%
36 MONTHS	5	100%	0	0%	0	0%	0	0%

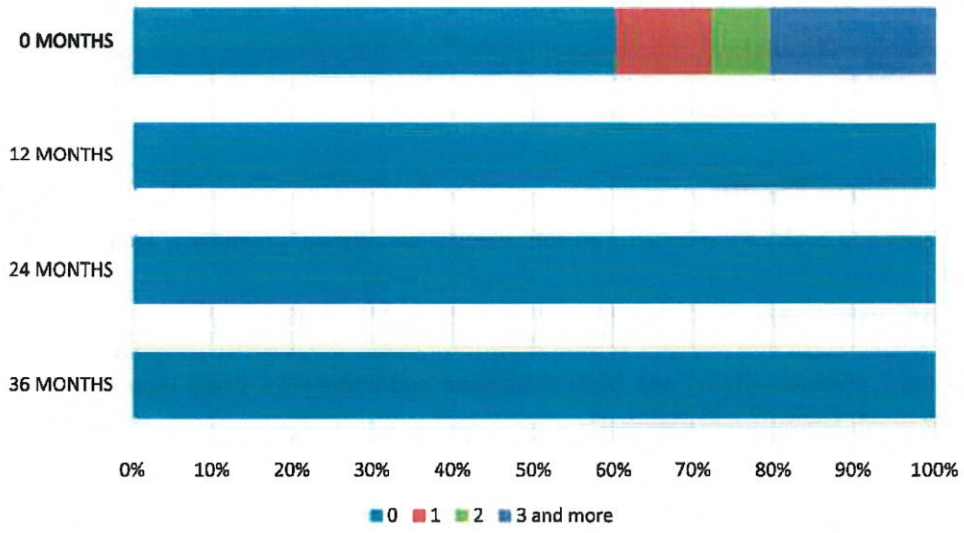

Figure 30: Bar chart of number of active Gd-enhancing lesions during the study

9. SAFETY RESULTS

All spontaneously reported AEs received through the study regardless of whether the event was serious, labeled, or attributed to Tysabri® were reported to regulatory authorities in accordance with 21 CFR 314.80 in the US and Regulation (EC) No. 726/2004 and Directive 2001/89/E as amended and Volume 9a of the Rules Governing Medicinal Products in the European Union in the E.U.

A long-term safety assessment of the Tysabri® treatment was performed as the review of SAEs/AEs, positivity of JCV and NSQ regarding to monitoring PML.

All SAEs and related AEs were documented and collected. The investigators were responsible for forwarding all SAEs and AEs to the Pharmacovigilance vendor who was responsible for safety reporting. Safety assessment consists in a summary of SAEs and AEs obtained from the Pharmacovigilance vendor.

Presence of anti-JCV antibodies was tested in baseline. If positive, subjects were closely monitored on PML symptoms during the course of the study. If negative, subjects had to be tested every 12 months.

NSQ was completed by subjects prior to every Tysabri® infusion administration in order to capture symptoms of PML.

9.1. Adverse Events

Individual AEs and SAEs were coded into four categories: relapse, pregnancy, lack of efficacy, and other.

AEs and SAEs were recorded in 104 subjects (AE 72%, SAE 27 %) as described in the Table 42. The most frequent event was Nervous system disorders (36 %), followed by Respiratory, thoracic and mediastinal disorders (16%) and Musculoskeletal and connective tissue disorders (8%) as summarized in the Table 42 and Figure 31.

Table 42: Types of events

	AE		SAE		N/A		SUM	
	n	%	n	%	n	%	n	%
Nervous system disorders	21	20.2%	16	15.4%	0	0.0%	37	35.6%
Respiratory, thoracic and mediastinal disorders	14	13.5%	3	2.9%	0	0.0%	17	16.3%
Musculoskeletal and connective tissue disorders	5	4.8%	3	2.9%	0	0.0%	8	7.7%
General disorders and administration site conditions	7	6.7%	0	0.0%	0	0.0%	7	6.7%
Skin and subcutaneous tissue disorders	6	5.8%	0	0.0%	0	0.0%	6	5.8%
Infections and infestations	4	3.8%	1	1.0%	0	0.0%	5	4.8%
Gastrointestinal disorders	4	3.8%	0	0.0%	0	0.0%	4	3.8%
Renal and urinary disorders	4	3.8%	0	0.0%	0	0.0%	4	3.8%
Surgical and medical procedures	0	0.0%	3	2.9%	0	0.0%	3	2.9%
Immune system disorders	2	1.9%	0	0.0%	0	0.0%	2	1.9%
Injury, poisoning and procedural complications	2	1.9%	0	0.0%	0	0.0%	2	1.9%
Pregnancy, puerperium and perinatal conditions	1	1.0%	0	0.0%	1	1.0%	2	1.9%
Vascular disorders	2	1.9%	0	0.0%	0	0.0%	2	1.9%
Hepatobiliary disorders	0	0.0%	1	1.0%	0	0.0%	1	1.0%
Investigations	1	1.0%	0	0.0%	0	0.0%	1	1.0%
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1	1.0%	0	0.0%	0	0.0%	1	1.0%
Psychiatric disorders	0	0.0%	1	1.0%	0	0.0%	1	1.0%
Social circumstances	1	1.0%	0	0.0%	0	0.0%	1	1.0%
Total	75	72.1%	28	26.9%	1	1.0%	104	100.0%

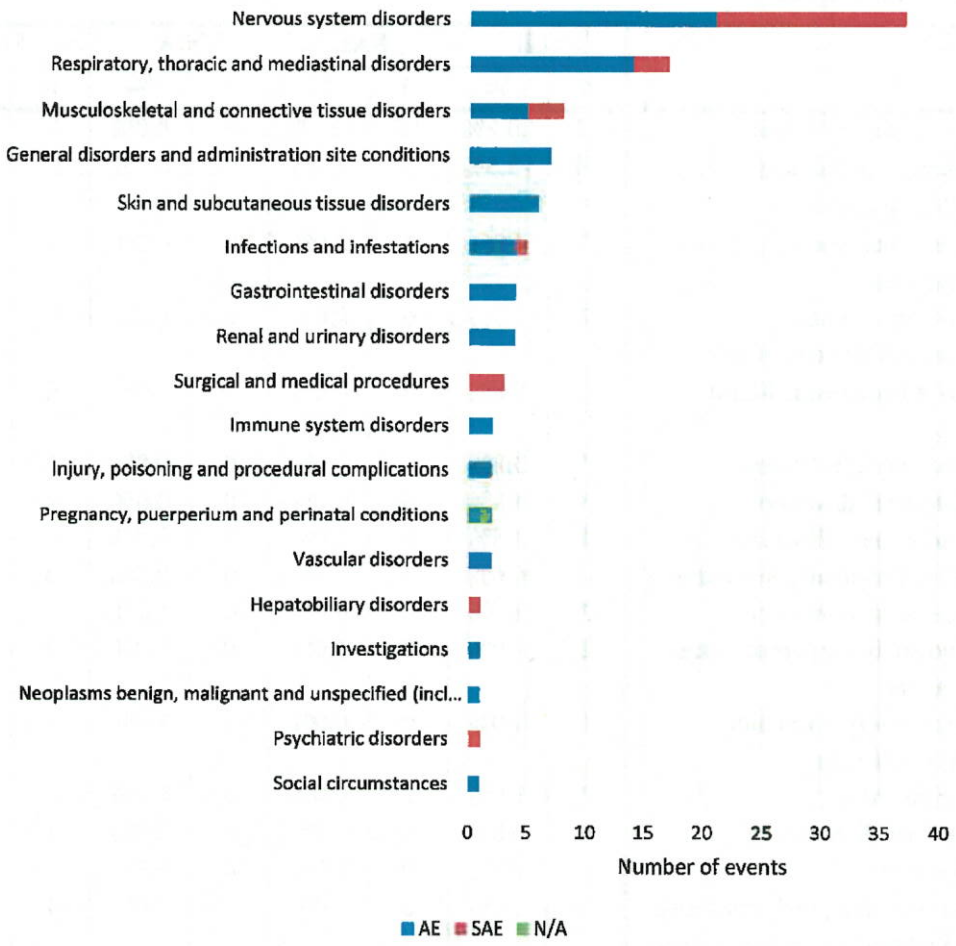

Figure 31: Bar chart of type of event

9.2. Other assessments

9.2.1. JCV

JCV-positivity mildly increased in last year (month 36) as described in the Table 43 and Figure 32; however, the number of patients in Month 24 and 36 is very low.

Table 43: Number of collected JC Virus-detecting tests

	positive		negative		missing
	N	%	N	%	N
JCV (0 MONTHS)	32	36%	58	64%	6
JCV (12 MONTHS)	19	39%	30	61%	3
JCV (24 MONTHS)	6	32%	13	68%	1
JCV (36 MONTHS)	5	63%	3	38%	1

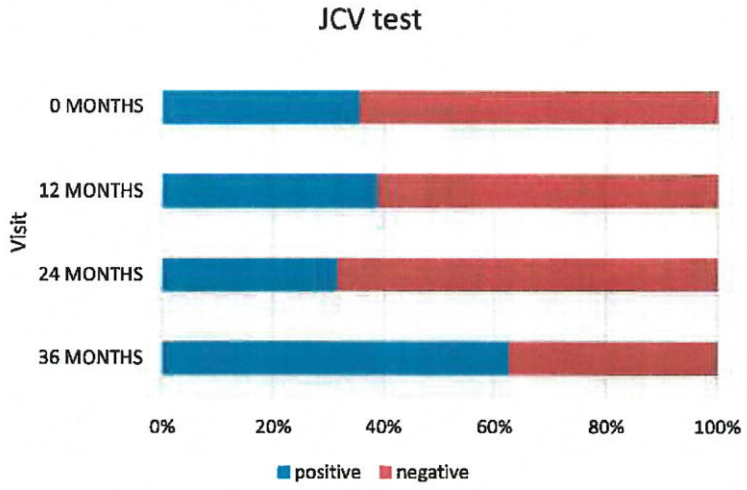

Figure 32: Number of positive/negative JC Virus-detecting tests

9.2.2. NSQ

NSQ is used as tool to monitoring of PML in patient with Tysabri® treatment.

NSQ has 12 yes/no questions related to health change from the last administration of Tysabri® infusion. It is non-standard questionnaire; therefore, every question is analysed separately.

List of questions:

1. Memory disorders
2. Memory recall disorders
3. Attention deficit disorders
4. Speech sound disorders
5. Limb weakness
6. Limb coordination
7. Walking difficulty
8. Visual disorders
9. Difficulty with balance
10. Frequent change in mood
11. Anxiety and depression
12. Spasticity of muscles

In NSQ, there was determined the most difficulty with limb weakness, walking, balance, and spasticity during the study. Figure 33 summarizes frequency of positive answers in NSQ during the study. The following tables and figures describe answers during the study to each NSQ question separately.

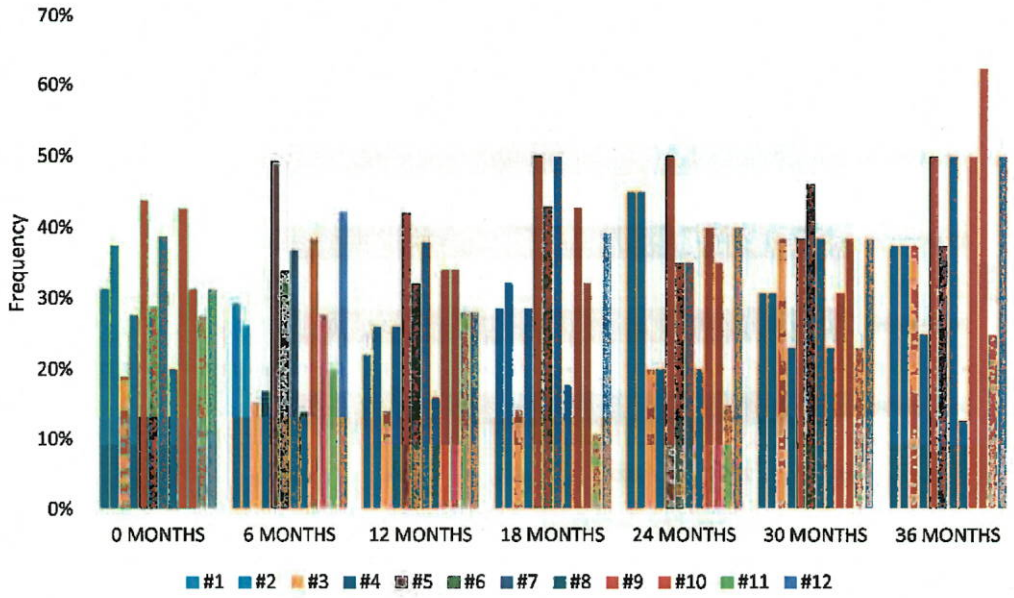

Figure 33: Frequency of positive answer in NSQ

Table 44: NSQ question #1 – Memory disorders

	yes		no		missing
	N	%	N	%	N
0 MONTHS	25	31%	55	69%	16
6 MONTHS	19	29%	46	71%	2
12 MONTHS	11	22%	39	78%	2
18 MONTHS	8	29%	20	71%	4
24 MONTHS	9	45%	11	55%	0
30 MONTHS	4	31%	9	69%	0
36 MONTHS	3	38%	5	63%	1

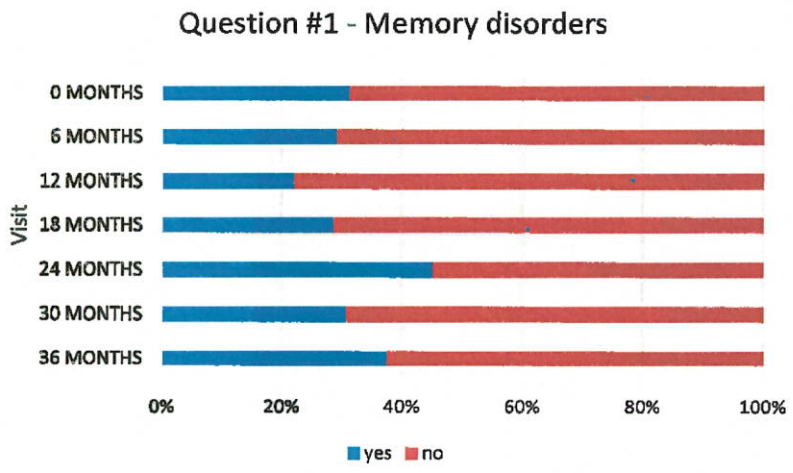

Figure 34: Bar chart of NSQ question #1 - Memory disorders

Table 45: NSQ question #2 – Memory recall disorders

	yes		no		missing
	N	%	N	%	N
0 MONTHS	30	38%	50	63%	16
6 MONTHS	17	26%	48	74%	2
12 MONTHS	13	26%	37	74%	2
18 MONTHS	9	32%	19	68%	4
24 MONTHS	9	45%	11	55%	0
30 MONTHS	4	31%	9	69%	0
36 MONTHS	3	38%	5	63%	1

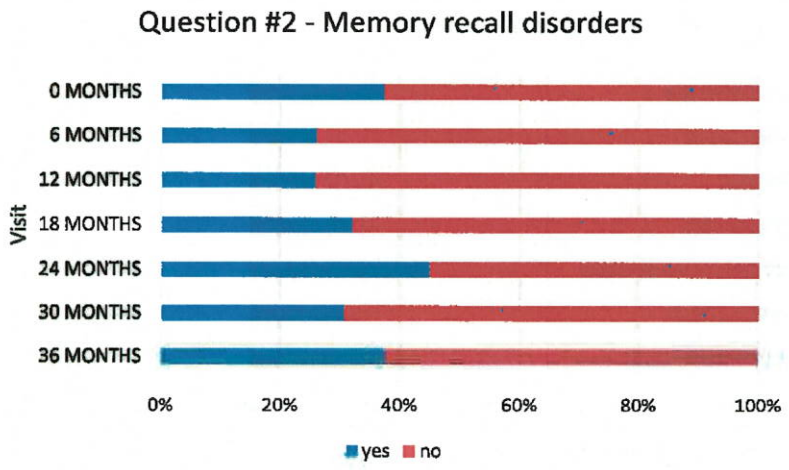

Figure 35: Bar chart of NSQ question #2 - Memory recall disorders

Table 46: NSQ question #3 – Attention deficit disorders

	yes		no		missing
	N	%	N	%	N
0 MONTHS	15	19%	64	81%	17
6 MONTHS	10	15%	55	85%	2
12 MONTHS	7	14%	43	86%	2
18 MONTHS	4	14%	24	86%	4
24 MONTHS	4	20%	16	80%	0
30 MONTHS	5	38%	8	62%	0
36 MONTHS	3	38%	5	63%	1

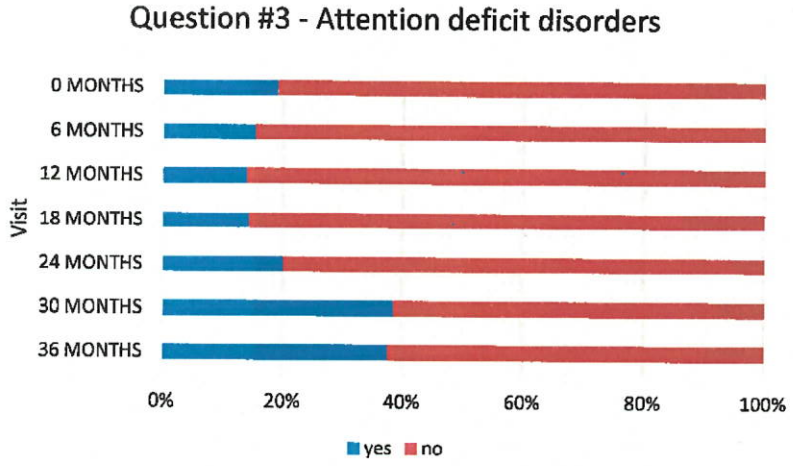

Figure 36: Bar chart of NSQ question #3 – Attention deficit disorders

Table 47: NSQ question #4 – Speech sound disorders

	yes		no		missing
	N	%	N	%	N
0 MONTHS	22	28%	58	73%	16
6 MONTHS	11	17%	54	83%	2
12 MONTHS	13	26%	37	74%	2
18 MONTHS	8	29%	20	71%	4
24 MONTHS	4	20%	16	80%	0
30 MONTHS	3	23%	10	77%	0
36 MONTHS	2	25%	6	75%	1

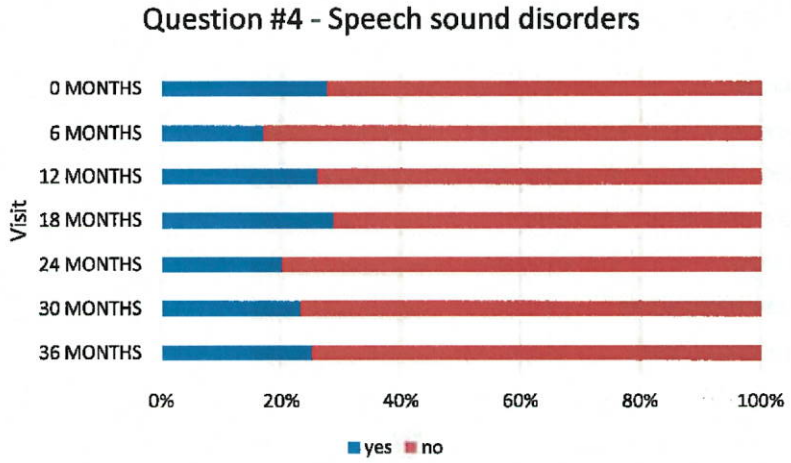

Figure 37: Bar chart of NSQ question #4 – Speech sound disorders

Table 48: NSQ question #5 –Limb weakness

	yes		no		missing
	N	%	N	%	N
0 MONTHS	35	44%	45	56%	16
6 MONTHS	32	49%	33	51%	2
12 MONTHS	21	42%	29	58%	2
18 MONTHS	14	50%	14	50%	4
24 MONTHS	10	50%	10	50%	0
30 MONTHS	5	38%	8	62%	0
36 MONTHS	4	50%	4	50%	1

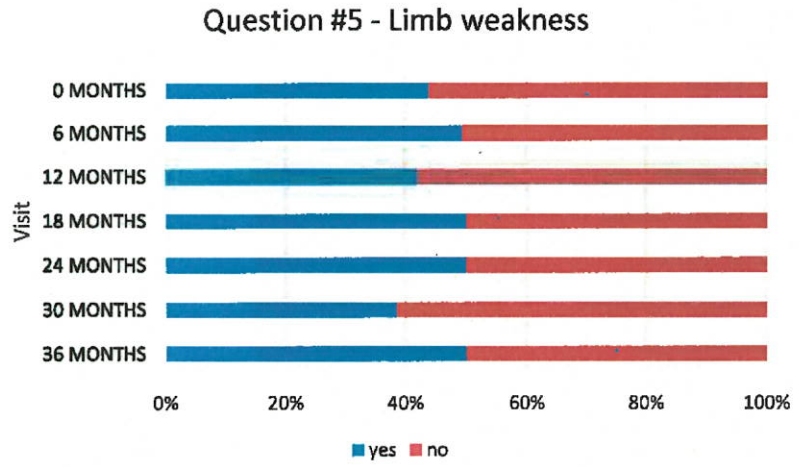

Figure 38: Bar chart of NSQ question #5 – Limb weakness

Table 49: NSQ question #6 –Limb coordination disorder

	yes		no		missing
	N	%	N	%	N
0 MONTHS	23	29%	57	71%	16
6 MONTHS	22	34%	43	66%	2
12 MONTHS	16	32%	34	68%	2
18 MONTHS	12	43%	16	57%	4
24 MONTHS	7	35%	13	65%	0
30 MONTHS	6	46%	7	54%	0
36 MONTHS	3	38%	5	63%	1

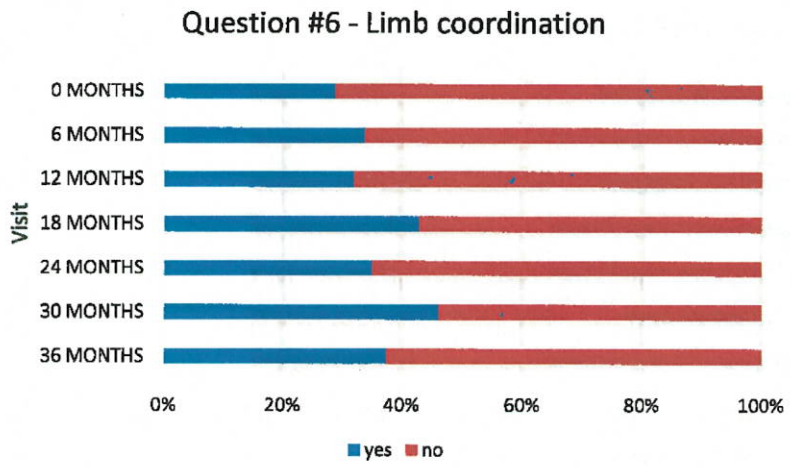

Figure 39: Bar chart of NSQ question #6 – Limb coordination disorder

Table 50: NSQ question #7 – Walking difficulty

	yes		no		missing
	N	%	N	%	N
0 MONTHS	31	39%	49	61%	16
6 MONTHS	24	37%	41	63%	2
12 MONTHS	19	38%	31	62%	2
18 MONTHS	14	50%	14	50%	4
24 MONTHS	7	35%	13	65%	0
30 MONTHS	5	38%	8	62%	0
36 MONTHS	4	50%	4	50%	1

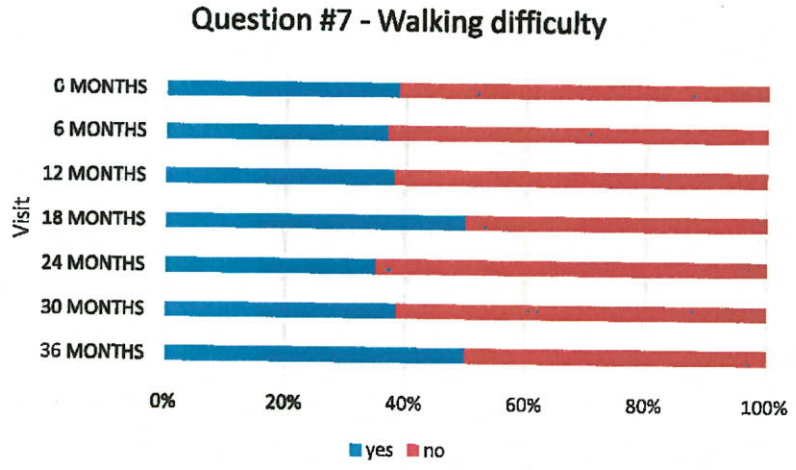

Figure 40: Bar chart of NSQ question #7 – Walking difficulty

Table 51: NSQ question #8 – Visual disorders

	yes		no		missing
	N	%	N	%	N
0 MONTHS	16	20%	64	80%	16
6 MONTHS	9	14%	56	86%	2
12 MONTHS	8	16%	42	84%	2
18 MONTHS	5	18%	23	82%	4
24 MONTHS	4	20%	16	80%	0
30 MONTHS	3	23%	10	77%	0
36 MONTHS	1	13%	7	88%	1

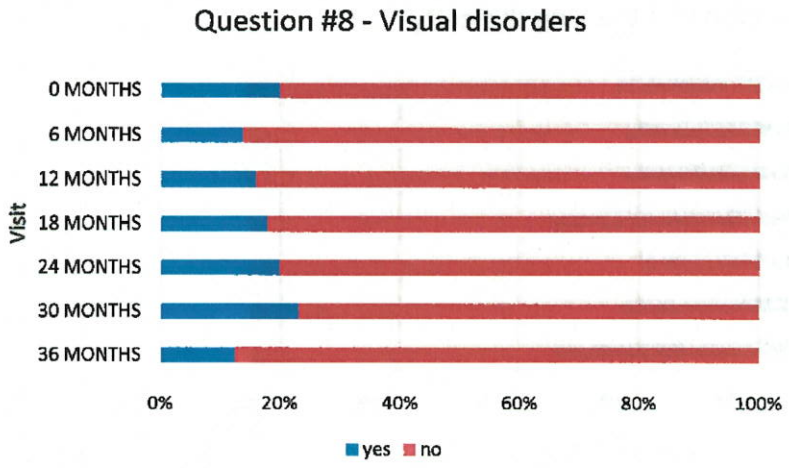

Figure 41: Bar chart of NSQ question #8 - Visual disorders

Table 52: NSQ question #9 – Difficulties with balance

	yes		no		missing
	N	%	N	%	N
0 MONTHS	34	43%	46	58%	16
6 MONTHS	25	38%	40	62%	2
12 MONTHS	17	34%	33	66%	2
18 MONTHS	12	43%	16	57%	4
24 MONTHS	8	40%	12	60%	0
30 MONTHS	4	31%	9	69%	0
36 MONTHS	4	50%	4	50%	1

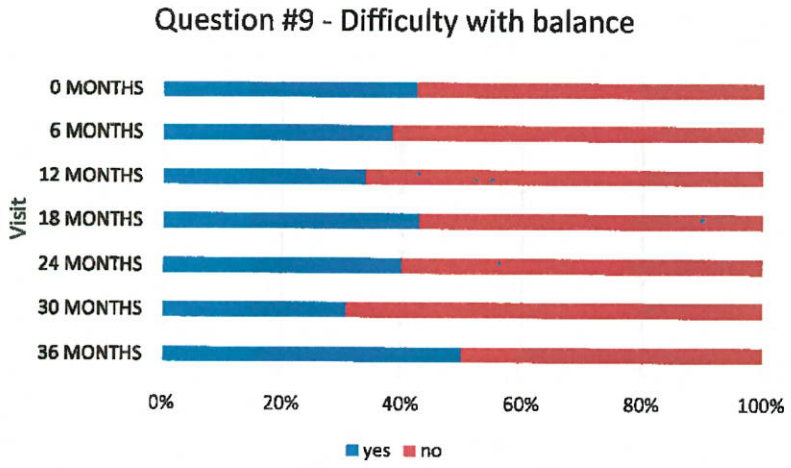

Figure 42: Bar chart of NSQ question #9 – Difficulties with balance

Table 53: NSQ question #10 – Frequent change in mood

	yes		no		missing
	N	%	N	%	N
0 MONTHS	25	31%	55	69%	16
6 MONTHS	18	28%	47	72%	2
12 MONTHS	17	34%	33	66%	2
18 MONTHS	9	32%	19	68%	4
24 MONTHS	7	35%	13	65%	0
30 MONTHS	5	38%	8	62%	0
36 MONTHS	5	63%	3	38%	1

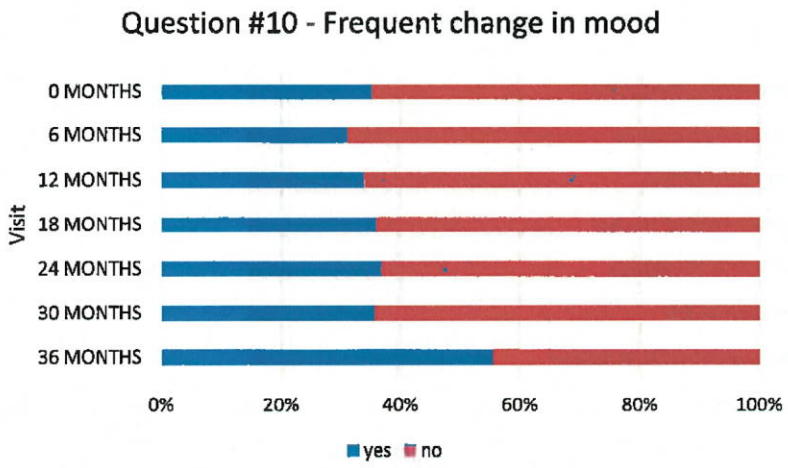

Figure 43: Bar chart of NSQ question #10 – Frequent change in mood

Table 54: NSQ question #11 – Anxiety and depression

	yes		no		missing
	N	%	N	%	N
0 MONTHS	22	28%	58	73%	16
6 MONTHS	13	20%	52	80%	2
12 MONTHS	14	28%	36	72%	2
18 MONTHS	3	11%	25	89%	4
24 MONTHS	3	15%	17	85%	0
30 MONTHS	3	23%	10	77%	0
36 MONTHS	2	25%	6	75%	1

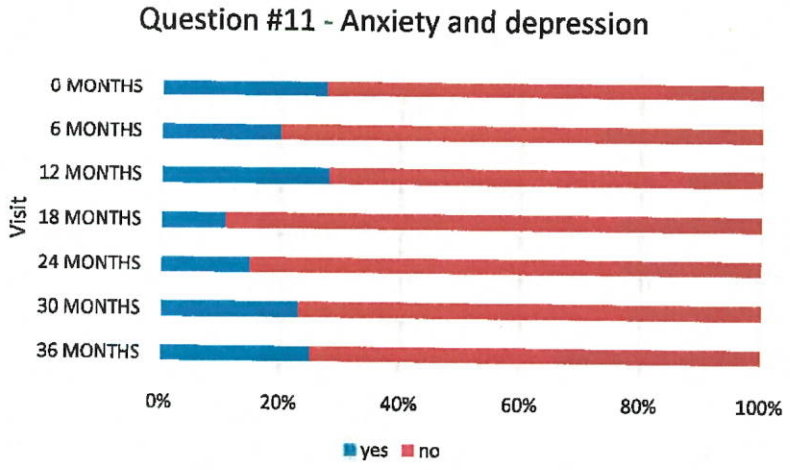

Figure 44: Bar chart of NSQ question #11 – Anxiety and depression

Table 55: NSQ question #12 – Spasticity of muscles

	yes		no		missing
	N	%	N	%	N
0 MONTHS	25	31%	55	69%	16
6 MONTHS	27	42%	37	58%	3
12 MONTHS	14	28%	36	72%	2
18 MONTHS	11	39%	17	61%	4
24 MONTHS	8	40%	12	60%	0
30 MONTHS	5	38%	8	62%	0
36 MONTHS	4	50%	4	50%	1

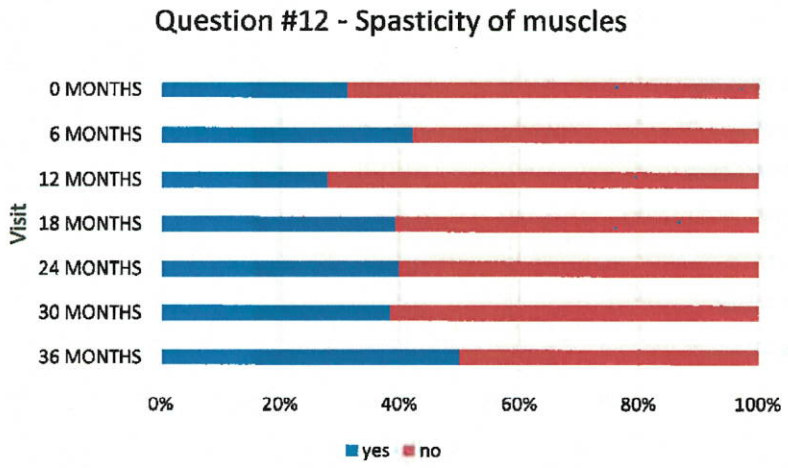

Figure 45: Bar chart of NSQ question #12 – Spasticity of muscles

10. DISCUSSION AND OVERALL CONCLUSIONS

Despite several measures taken to strengthen the recruitment, including the addition of other countries, was non-interventional clinical trial LATYSS prematurely terminated by the sponsor Biogen Slovakia, Ltd. on 27 November 2015. This was due to significant delay in patients' recruitment.

Patients with MS frequently report poor sleep, and sleep disorders are more common in MS patients compared to healthy controls (Merlino et al., 2009.) Sleep disturbances can contribute to depression, pain and fatigue – symptoms that are commonly seen in MS patients and that are often disabling.

There are several limitations of the present study that need to be addressed. The most important limitation is that the total number of 200 enrolled patients was not reached in Slovakia. Even though recruitment had been open for patients in Hungary and Poland during the year 2014, planned target number of patients was not reached. As a result of slow patient enrolment, study was prematurely terminated by sponsor on 27th November 2015. According to investigators, the main reason of slow patient recruitment was the low number of eligible patients. No safety issue was involved in the insufficient patient enrolment rate or in the study termination by sponsor. The total count of patients, whose data were used for analysis of study objectives, is 96 although end of study visit is available in 91 patients (5 withdrawn patients have unavailable EOS page of CRF). Furthermore, there is decreasing number of patients who completed subsequent visits before the study termination. Finally, there are only 9 patients with valid assessments for visit month 36. To avoid mistakes resulting from the small number of participating subject in later visits, primary endpoint analysis – change of PSQI from baseline – was performed to visits Month 6 and 12 only.

Another limitation involves use of self-reported questionnaires as the study endpoints. Scores can be easily skewed by the person completing them with exaggerating or minimizing his/her subjective assessment of the investigated health issue. However, these tools offer insight into patients' view and feelings that are also very important factors for the assessment of successful treatment.

The primary objective of the study, PSQI is a self-rated questionnaire, which assesses sleep quality and disturbances by seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction). Baseline PSQI in the present study population (7.4) is in accordance with recently published PSQI in MS population (7.7) (Veauthier et al., 2016).

Positive effect on sleep quality during Tysabri® treatment assessed by PSQI change from baseline was confirmed at visit in Month 6 ($P = 0.004$, $n = 67$, 95% CI (-1.76 to -0.36)). No statistical significant change was observed between baseline and Month 12 [$P = 0.378$, $n = 52$, 95% CI (-1.13 to 0.40)]. Number of observations at month 18, 24, 30 and 36 are low and the results could be irrelevant.

No correlation was detected using Spearman correlation coefficient between PSQI and the number of T2 lesions.

MFIS shows slight improvement during the study, although data from month 30 and 36 had higher variability most probably due to the lower number of evaluated patients. Recently published study (Wilken et al, 2013) describes significant improvement of MFIS after 12 months of natalizumab

treatment (P < .0001) in patients with the evidence of fatigue at baseline (mean baseline MFIS score 59.1, while mean baseline MFIS score in the present study was 36.6).

ESS shows the similar improvement pattern as MFIS. Especially data from month 30 and 36 were again collected from lower number of patients compared to the other time points.

EQ-5D-3L and EQ-VAS did not detect any change of quality of life during the study. According to previous experience, quality of life increases during the natalizumab treatment (Stephenson et al., 2012; Kamat et al., 2009; Rudick et al., 2007).

During the study, BDI-II shows improving trend, however values from month 30 and 36 could be influenced by the small number of evaluated patient. Recent study (Edwards et al., 2016) describes improvement of BDI-II by 2.45 points (P = 0.001) after month 6 of natalizumab treatment. For comparison, both used BDI-II versions scores dropped by 2.0 points after 6 months in the present study.

ARR was clearly improved during the treatment with Tysabri®. This finding is in accordance with the latest published study of natalizumab efficacy (Spelman et al., 2016) likewise with the results of Tysabri® pivotal clinical study AFFIRM (Polman et al., 2006).

EDSS was assessed every 6 months. During the study, the score was varying with slightly increasing trend. The median value remained nearly the same for the whole study (3.0-4.0). This is in accordance with result from extensive Danish study (Koch-Henriksen et al., 2016), extensive 5years Tysabri® Observational Program TOP (Butzkueven et al., 2014) and (Mattioli et al., 2015), where EDSS were stable during natalizumab treatment.

No change of MoCA score was detected in the study, although the improvement of cognitive function during natalizumab treatment was previously detected (Mattioli et al., 2015).

There were 62% economically active subjects at baseline and 51% at end of study. Part-time resp. full-time employment was observed in 18% resp. 83% at baseline and in 19% resp. 81% at end of study. Manual resp. intellectual employment was recorded in 27% reps. 73% at baseline and in 20% resp. 80% at end of study. Hence, there was no deterioration of ability to work during the Tysabri® treatment.

Number of T2 lesions increased during the treatment, however highest values from month 24 and 36 are accompanied with the lower number of assessed patients. In the study assessing MRI parameters during natalizumab treatment (Khalid et al., 2016), no progression of cerebral T2 hyperintense lesion volume was detected during the observation period. Furthermore, the number of active Gd lesions, the second MRI parameter assessed in the present study, considerably dropped during Tysabri® treatment suggesting the improvement of patient status.

The one of the most serious safety concern of natalizumab is PML and patients had to fill NSQ during Tysabri® treatment to capture symptoms of PML. In NSQ evaluated during the present study, there was determined the most difficulty with limb weakness, walking, balance, and spasticity. No clear trend was detected in functions monitored by NSQ during the study. JCV positivity mildly increased during the study; however, no PML was reported during the study.

There were recorded 104 AEs and SAEs (AE 72%, SAE 27 %). The most frequent events were Nervous system disorders (36%), then Respiratory, thoracic and mediastinal disorders (17%) and Musculoskeletal and connective tissue disorders (7%). In the pivotal Tysabri® clinical trial AFFIRM, 95% of participating subjects receiving Tysabri® reported at least one AE and 19% of

patients experienced SAE. However, there was at least one AE in 96% of subjects receiving placebo and 24% of patients from placebo group experienced SAE (Polman et al., 2006). If we review the reasons for the withdrawal in the present study, there is only a few of cases possibly attributable to the therapy adverse events: 4 patients for lack of efficacy, 2 patients for allergic reaction to Tysabri® and 1 patient with fulminant hepatitis. Finally, Tysabri® SPC states that 43.5% of patients treated with natalizumab reported adverse reactions as a result from over 2-years long placebo-controlled trials and that adverse events leading to discontinuation of therapy occurred in 5.8% of patients treated with natalizumab.

In conclusion, the present study confirmed significant effect of Tysabri® treatment on relapse rate reduction and stabilization of patients' clinical status. The other evaluated self-reported endpoints improved or remained stable during the followed three years of Tysabri® treatment.

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12. APPENDICES

APPENDIX A. LISTING OF ETHICS COMMITTEES

APPENDIX B. LISTING OF INVESTIGATORS

APPENDIX C. STATISTICAL ANALYSIS PLAN

APPENDIX D. INVESTIGATOR SIGNATURE PAGE

APPENDIX A. LISTING OF ETHICS COMMITTEES

Site No.	Investigator / Institution Name	Name / Address of Ethics Committee
SK01-05	Multicenter ethic committee	Etická komisie Bratislavského samosprávneho kraja/ Sabinovská 16, P.O. Box 106 820 05 Bratislava 25
SK01	Prof. MUDr. Peter Turčáni, PhD. / I. Neurologická klinika UNB Nemocnica Staré Mesto Mickiewiczova 13 813 69 Bratislava	Etická komisie UNB Nemocnica Staré Mesto Mickiewiczova 13 813 69 Bratislava
SK02	Prof. MUDr. Ľubomír Lisý, DrSc./ Neurologická klinika UNB Nemocnica Ružinov Ružinovská 6 826 06 Bratislava	Etická komisia UNB Nemocnica Ružinov/ Ružinovská 6, 826 06 Bratislava
SK03	MUDr. Ľubica Procházková, CSc./IL Neurologická klinika UNB Nemocnica akad. L. Dérera Limbová 5 833 05 Bratislava	Etická komisia UNB Nemocnica ak. L. Dérera/ Limbová 5 833 05 Bratislava
SK04	Doc. MUDr. Vladimír Donáth, PhD./ Neurologická klinika FNsP F.D. Roosevelta Námestie L. Svobodu 1 975 17 Banská Bystrica	Etická komisia FNsP F.D. Roosevelta/ Nám. L. Svobodu 1 975 17BanskáBystrica
SK05	MUDr. Anna Šaffová/Neurologická klinika ÚVN SNP Ružomberok – FN Generála MilošaVesela 21 034 26 Ružomberok	Etická komisia UVN SNP Ružomberok –FN/ Generála MilošaVesela 21 034 26 Ružomberok
HU10-16	Multicenter ethic committee	Egészsgügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság (ETT TUKEB)/ Egészsegügyi Tudományos Tanács Titkárság Arany János u. 6-8 Budapest 1051

Site No.	Investigator / Institution Name	Name / Address of Ethics Committee
PL20-31	Multicenter ethic committee	Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku/ ul. Jana Kilińskiego 1 15-089 Białymstok

APPENDIX B. LISTING OF INVESTIGATORS

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SK02	Prof. MUDr. Ľubomír Lisý, DrSc.	Neurologická klinika UNB Nemocnica Ružinov Ružinovská 6 826 06 Bratislava	29
SK03	MUDr. Ľubica Procházková, CSc.	II. Neurologická klinika UNB Nemocnica akad. L. Dérera Limbová 5 833 05 Bratislava	13
SK04	Doc. MUDr. Vladimír Donáth, PhD.	Neurologická klinika FNsP F.D. Roosevelta Námestie L. Svobodu 1 975 17 Banská Bystrica	6
SK05	MUDr. Anna Šaffová	Neurologická klinika ÚVN SNP Ružomberok FN, Generála MilošaVesela 21 034 26 Ružomberok	6
HU10	Dr. Péter Diószeghy	Jósa András Hospital Szt. István u. 68. H-4400 Nyíregyháza	2
HU11	Dr. Piroska Imre	Csolnoky Ferenc Kórház Kórház út 1. H-8200 Veszprém	4
HU12	Dr. Csilla Rózsa	Jahn Ferenc Hospital Köves út 2-4. H-1204 Budapest	0
HU13	Dr. Krisztina Bencsik	University of Szeged Semmelweis u. 6. H-6725 Szeged	1

Site No.	Investigator Name	Institution and Address	Number of Subjects
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PL20	Dr hab. n. med. Alina Kułakowska	Uniwersytecki Szpital Kliniczny w Białymstoku ul. M. Skłodowskiej-Curie 24A 15-276 Białymstok	3
PL21	Prof. dr hab. med. Anna Członkowska	Instytut Psychiatrii i Neurologii ul. Sobieskiego 9 02-957 Warszawa	3
PL22	Dr Jacek Wencel	Zakład Opieki Zdrowotnej MSWiA w Poznaniu im. prof. L. Bierkowskiego ul. Dojazd 34 60-631 Poznań	4
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Site No.	Investigator Name	Institution and Address	Number of Subjects
PL27	Dr n. med. Waldemar Brola	Zespół Opieki Zdrowotnej w Końskich ul. Gimnazjalna 41B 26-200 Końskie	0
PL28	Prof. Krystyna Pierzchała	Samodzielny Szpital Kliniczny nr 1 im. Prof. Stanisława Szyszko Śląskiego Uniwersytetu Medycznego w Katowicach ul. 3-go Maja 13-15 41-800 Zabze	0
PL29	Prof. Monika Rudzińska	Samodzielny Publiczny Centralny Szpital Kliniczny im. Kornela Gibińskiego Śląskiego Uniwersytetu Medycznego w Katowicach ul. Medyków 14 40-752 Katowice	0
PL30	Prof. Bartłomiej Karaszewski	Uniwersyteckie Centrum Kliniczne ul. Dębinki 7 80-952 Gdańsk	0
PL31	Dr Monika Marona	Samodzielny Publiczny Zakład Opieki Zdrowotnej Szpital Uniwersytecki w Krakowie ul. Mikołaja Kopernika 19 31-000 Kraków	2

APPENDIX C. STATISTICAL ANALYSIS PLAN

No statistical analysis plan was created for this study.

APPENDIX D. INVESTIGATOR SIGNATURE PAGE

Investigator Signature Page

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of Study<XXX>.

_____ 10/FEB/2017

<Principal/Coordinating> Investigator's Signature Date

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<Principal/Coordinating> Investigator's Affiliation:

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