**Supplementary Appendix 1**

**(Study cohorts and clinical evaluation)**

**Patients cohorts**

*ASA cohort*

The ASA study investigated clinical and MRI outcomes among patients with RRMS treated with intramuscular interferon-beta-1a (IM IFNß-1a) (Avonex 30 mg IM weekly; Biogen Idec, Weston, MA, USA).1 The original ASA study was a 2-year randomized, double-blind, placebo-controlled trial with a 5-year open-label extension. Subjects were recruited from one MS center in the Czech Republic between years 1999 and 2003. Inclusion criteria were as follows: age 18–55 years, clinically definite RRMS confirmed by MRI, ≥2 oligoclonal bands in the CSF, EDSS≤3.52 and ≥2 relapses over the past 12 months or ≥3 relapses over the past 24 months. The patients in the SET study were originally diagnosed with clinically isolated syndrome (CIS) based on to the 2005 McDonald criteria, with later reclassification using the 2017 McDonald criteria.3 Patients in the extension study were maintained on the study treatment assigned at baseline, as per the standard follow-up protocol.

Patients were randomized in a 1:1:1 ratio to one of three treatment groups: IM IFNβ-1a 30 μg once weekly plus placebo azathioprine (AZA) orally (p.o.) once daily, plus placebo corticosteroid p.o. every other day; IM IFNβ-1a 30 μg once weekly plus AZA 50 mg p.o. once daily, plus placebo corticosteroid p.o. every other day; or IM IFNβ-1a 30 μg once weekly plus AZA 50 mg p.o. once daily, plus prednisone 10 mg p.o. every other day. Patients were randomized at the baseline visit after all eligibility criteria were confirmed. Randomization was stratified by EDSS score, age, and gender using a centralized randomization schedule to balance the treatment group assignments.

After the end of the study, the patients were followed-up in routine clinical practice (clinical and MRI data collected until 11/2015).

*SET*

The SET (Study of Early Interferon b1-a Treatment in High Risk Subjects after CIS; EudraCT identification number 2005-001281-13) study investigated patients after the first demyelinating event suggestive of MS. It was a 2-year investigator initiated, prospective, observational clinical study of IM IFNß-1a treatment (clin.gov #NCT01592474) in the Czech Republic.4, 5 Subjects were recruited from 8 MS centres within the Czech Republic between years 2005 and 2009. The study database was locked in 8/2011.The inclusion criteria were as follows: age 18–55 years, enrollment within 4 months from the disease onset, Expanded Disability Status Scale (EDSS)2 score of ≤3.5; ≥2 T2-hyperintense lesions on diagnostic brain MRI and ≥2 oligoclonal bands in the cerebrospinal fluid (CSF). Exclusion criteria were: second relapse before enrolment, any major disease or pregnancy. The patients in the SET study were originally diagnosed with clinically isolated syndrome (CIS) based on to the 2005 McDonald criteria, with later reclassification using the 2017 McDonald criteria.3

After the end of the study, the patients were followed-up in routine clinical practice (clinical and MRI data collected until 11/2015).

*QMRI cohort*

All MS patients routinely followed at the MS Centre at the General University Hospital in Prague, and with brain MRI after 3/2000 until 11/2015, were included in the QMRI program.6 The inclusion criteria were as follows: age>18 years, assessment of CSF and diagnosis of MS.

**Clinical evaluation**

After the SET and ASA study completion, patients received standard clinical follow-up, consisting of 3- to 6-monthly visits, similarly to the QMRI cohort. Confirmed disability worsening was defined as worsening by an increase in the EDSS of 1.5 points if the baseline EDSS score was 0; 1.0 point if the baseline EDSS score was between 1.0 and 5.0; and 0.5 point for baseline EDSS scores of 5.5 or higher.7 We required confirmation of disability worsening after 12 months. Baseline EDSS was established using the median of the EDSS score during the first 6 months of follow-up.

**References:**

1. Havrdova E, Zivadinov R, Krasensky J, et al. Randomized study of interferon beta-1a, low-dose azathioprine, and low-dose corticosteroids in multiple sclerosis. Mult Scler 2009;15:965-976.

2. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444-1452.

3. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17:162-173.

4. Kalincik T, Vaneckova M, Tyblova M, et al. Volumetric MRI markers and predictors of disease activity in early multiple sclerosis: a longitudinal cohort study. PLoS One 2012;7:e50101.

5. Uher T, Horakova D, Bergsland N, et al. MRI correlates of disability progression in patients with CIS over 48 months. Neuroimage Clin 2014;6:312-319.

6. Uher T, Vaneckova M, Krasensky J, et al. Pathological cut-offs of global and regional brain volume loss in multiple sclerosis. Mult Scler 2019;25:541-553.

7. Kalincik T, Cutter G, Spelman T, et al. Defining reliable disability outcomes in multiple sclerosis. Brain 2015;138:3287-3298.