Supplementary Materials

Appendix 1: Supplementary methodological details

IFN beta-1a BAb assay

The IFN beta-1a BAb assay captures serum antibodies via an indirect coat of IFN beta-1a via murine monoclonal antibody specific for human IFN beta. Any captured anti-IFN beta-1a antibodies were subsequently detected by addition of a horseradish peroxidase (HRP)-labeled polyclonal anti-human IgG, followed by 3,3',5,5'-tetramethylbenzidine (TMB) substrate for colorimetric development. The color development was proportional to the anti-IFN β-1a antibody concentration in the serum. Samples with signals above a statistically derived cut point were designated positive.

Peginterferon beta-1a NAb assay

The peginterferon beta-1a NAb assay evaluates the ability of serum samples that tested positive for anti-IFN beta-1a antibodies in the screening ELISA to inhibit IFN beta activation of the type I interferon receptor (IFNAR) in a cell-based activity assay, specifically the induction of myxovirus resistance protein A (MxA) expression. Samples were added to A549 cells along with a fixed concentration of peginterferon beta-1a. NAbs against peginterferon beta-1a block IFN beta activation of IFNAR and reduce the MxA expression. Following incubation, A549 cell lysates were tested in an ELISA that detects MxA protein using colorimetric detection, which was proportional to MxA concentration in the cell lysates.

Anti-PEG Ab assay

The anti-PEG Ab assay captures serum antibodies on plates coated with PEG. Captured anti-PEG antibodies were subsequently detected by addition of a HRP-labeled goat anti-human IgG/IgM polyclonal antibody, followed by TMB substrate for colorimetric development. The color

development was proportional to anti-PEG antibody concentration in the serum. Samples with signals above a statistically derived cut point were retested with and without excess PEG to confirm positives, and then titrated to differentiate strength of signal.

Supplementary Table 1. Incidence of anti-peginterferon beta-1a NAbs over time

Year 1	**Placebo**		**Peginterferon beta-1a 125 mcg**	
			Every 2 weeks	Every 4 weeks
n[a]	499		513	500
Week 8	2/468 (<1)		0/475	0/474
Week 20	1/464 (<1)		0/453	0/461
Week 36	1/457 (<1)		3/450 (<1)	2/455 (<1)
Week 48	1/456 (<1)		3/441 (<1)	0/441
Any time in Year 1	*2/489 (<1)*		*4/491 (<1)*	*2/492 (<1)*
Year 2	**Delayed treatment**		**Peginterferon beta-1a 125 mcg**	
	Every 2 weeks	Every 4 weeks	Every 2 weeks	Every 4 weeks
n[b]	228	227	438	439
Week 60	0/221	1/219 (<1)	3/418 (<1)	0/424
Week 72	1/216 (<1)	0/214	4/420 (<1)	2/421 (<1)
Week 96	0/203	1/199 (<1)	4/415 (<1)	0/399
Any time in Year 2	*1/225 (<1)*	*2/223 (<1)*	*5/429 (1)*	*2/435 (<1)*

[a]Safety population

[b]Safety population dosed in Year 2

NAb: neutralizing antibody.

Supplementary Table 2. Secondary endpoints by antibody status[a] at Year 1 and over 2 years[b]

		Year 1					Over 2 years			
		Placebo (*n*=500)	**Peginterferon beta-1a every 2 weeks (*n*=512)**		**Peginterferon beta-1a every 4 weeks (*n*=500)**		**Peginterferon beta-1a every 2 weeks (*n*=438)**		**Peginterferon beta-1a every 4 weeks (*n*=438)**	
			Never positive	**Ever positive**	**Never positive**	**Ever positive**	**Never positive**	**Ever positive**	**Never positive**	**Ever positive**
Anti-IFN BAbs	*n*	500	458	54	472	28	384	54	403	35
New/newly enlarging T2 lesions, mean (SD)		13.3 (19.51)	3.9 (8.11)	5.3 (11.44)	9.5 (16.18)	5.2 (7.50)	5.7 (12.20)	6.0 (11.54)	15.0 (24.37)	13.1 (18.20)
Relapse-free patients,[c] *n* (%)		358 (72)	375 (82)	47 (87)	370 (78)	25 (89)	284 (74)	43 (80)	262 (65)	28 (80)
Disability progression-free patients,[d] *n* (%)		450 (90)	431 (94)	50 (93)	444 (94)	25 (89)	345 (90)	47 (87)	352 (87)	31 (89)
Anti-peginterferon NAbs	*n*	500	500	12	496	4	427	11	433	5
New/newly enlarging T2 lesions, mean (SD)		13.3 (19.51)	4.1 (8.60)	4.7 (6.12)	9.2 (15.88)	9.7 (10.26)	5.7 (12.04)	9.1 (15.35)	14.7 (23.97)	23.0 (17.66)

Relapse-free patients,[c] *n* (%)		358 (72)	410 (82)	12 (100)	391 (79)	4 (100)	318 (74)	9 (82)	286 (66)	4 (80)
Disability progression-free patients,[d] *n* (%)		450 (90)	469 (94)	12 (100)	465 (94)	4 (100)	382 (89)	10 (91)	378 (87)	5 (100)
Anti–PEG Abs	*n*	500	456	56	430	70	388	50	378	60
New/newly enlarging T2 lesions, mean (SD)		13.3 (19.51)	4.1 (8.76)	4.4 (6.77)	8.9 (15.19)	11.4 (19.40)	5.7 (12.33)	6.5 (10.49)	14.2 (23.57)	18.7 (25.84)
Relapse-free patients,[c] *n* (%)		358 (72)	374 (82)	48 (86)	339 (79)	56 (80)	289 (74)	38 (76)	252 (67)	38 (63)
Disability progression-free patients,[d] *n* (%)		450 (90)	428 (94)	53 (95)	399 (93)	70 (100)	349 (90)	43 (86)	324 (86)	59 (98)

[a]'Never positive' indicate patients without antibodies to peginterferon beta-1a, 'ever positive' indicates patients positive at any timepoint, including baseline.

[b]Patients who were treated with peginterferon beta-1a in both Year 1 and Year 2.

[c]Relapses defined as new or recurrent neurologic symptoms not associated with fever or infection, lasting for ≥24 hours, and accompanied by new objective neurologic findings, and separated from the onset of other confirmed relapses by ≥30 days, confirmed by the independent neurologic evaluation committee.

[d]Disability progression defined as an increase in the Expanded Disability Status Scale score of ≥1.0 point in patients with a baseline score of ≥1.0 or an increase of ≥1.5 points in patients with a baseline score of 0, confirmed after 12 weeks.

Ab: antibody; BAb: binding antibody; NAb: neutralizing antibody; SD: standard deviation.