APPENDIX

Protocol amendments

The study protocol was amended 19 times. All amendments were made before database lock. The key features of each amendment are given below.

Amendment 1 (14-Jul-2011), introduced the following changes:

- Addition of Exclusion Criterion 14: 'Patients with severe hepatic impairment (Child-Pugh class C)'.
- Addition of Australian sites, leading to an increase in the sample size.

Amendment 2 (03-Oct-2011), introduced the following change:

- New sites submitted in Spain (Hospital Reina Sofía de Córdoba, Hospital U. Nuestra Sra. De Candelaria, Hospital Germans Trias i Pujol, Hospital Clínico de Valladolid).

Amendment 3 (04-Nov-2011), introduced the following change:

- Change of Principal Investigator in Hospital Central de Asturias (Spain).

Amendment 4 (01-Dec-2011), introduced the following changes:

- A secondary endpoint was modified adding: 'Since not all participating centres have the SIENAX software, the analysis of all MRIs will be applicable only for a subpopulation of subjects'.
- Modification of the window between visits: 'The study will consist of four visits (V0, V1, V2 and V3) and a screening visit (V–1): Day –30 to –1, during which patients must be examined either at the scheduled visits or on the earliest day possible (day 0 for V0 and ±15 days for V1, V2 and V3)'.
- Inclusion criteria 6 was modified as follows:

  'Patients:

  a. Treatment naïve: patients who have never been treated with a DMT

  or

b. Previously treated with first-line DMT: patients who have been treated with a first-line DMT continuously for at least a 1-year period (interferon beta-1a [intramuscular or subcutaneous], interferon-beta-1b or glatiramer acetate)'

- Exclusion criteria 7 was modified as follows:
  'Patients who test negative for IgG antibodies against the varicella-zoster virus at the screening visit. Patients may be vaccinated once it is established that they have IgG antibodies and could be included at least 1 month after vaccination'.
- It included laboratory analysis at visit 1 of the study:
  'To assess safety, laboratory tests (haematology, biochemistry) will be conducted at the screening visit (V-1), visit 2 (24 weeks) and visit 3 (final study visit at 48 weeks or at early withdrawal from the study)'.

Amendment 5 (23-Jan-2012), introduced the following changes:

- Due to a new security measure from EMA, it added an ECG before medication administration and after 6 hours of administration. Arterial pressure and pulse frequency will be taken each hour until 6 hours.
- It added laboratory analysis at week 4 and at week 36.
- It included a series of remarks in the protocol.

Amendment 6 (29-May-2012), introduced the following changes:

- It has been specified that the inclusion criteria number 4 only applies to Spain.
- Added the inclusion criteria number 5, only applies to Australia.
- Added the exclusion criteria numbers 15 and 16.

Amendment 7 (03-Sep-2012), introduced the following change:

- New sites submitted in Spain (Hospital de Albacete and Hospital Santa Tecla).

Amendment 8 (01-Oct-2012), introduced the following changes:

- The centralization of MRI analysis of all patients in the trial in a central laboratory in Australia is included (Sydney Neuroimaging Analysis Centre, SNAC).
- They have made several clarifications on inclusion and exclusion criteria.

Amendment 9 (05-Nov-2012), introduced the following change:

- New sites submitted in Spain (Hospital Universitario 12 de Octubre).

Amendment 10 (03-Dec-2012), introduced the following change:

- New sites submitted in Spain (Hospital de León and Hospital Doctor Negrin).

Amendment 11 (08-Jan-2013), introduced the following change:

- Due to a security measure from EMA related to security requirements, it adds the same monitoring process as for treatment initiation when treatment is interrupted for:
    - 1 day or more during the first 2 weeks of treatment.
    - more than 7 days during weeks 3 and 4 of treatment.
    - more than 2 weeks after 4 weeks of treatment.

Amendment 12 (01-Mar-2013), introduced the following change:

- Change of Principal Investigator in Hospital Insular Gran Canaria (Spain).

Amendment 13 (04-Jul-2013), introduced the following change:

- Owing to several centres in Spain having difficulty adapting to the parameters of SNAC, SNAC analyse separately MRI of Australia and Spain. In addition, for patients in Spain, another MRI central analysis will be run at Institut de diagnòstic per la Imatge of Hospital Vall d'Hebron.

Amendment 14 (03-Jun-2014), introduced the following changes:

- The central analysis for patients in Spain at Institut de diagnòstic per la Imatge of Hospital Vall d'Hebron is deleted.
- Recruitment period is prolonged to December 2014.

Amendment 15 (10-Jun-2014), introduced the following change:

- Clarification on compliance checks in Australia, aligned with standard of care.

Amendment 16 (04-Jun-2015), introduced the following change:

- Change of Principal Investigator in Hospital Gregorio Marañón (Spain).

Amendment 17 (30-Oct-2015), introduced the following change:

- Clarification of some points in the protocol.
- Notification that an Interim Analysis was done.

Amendment 18 (03-Dec-2015), introduced the following change:

- Change of Principal Investigator in Hospital Carlos Haya (Spain).

Amendment 19 (04-Feb-2016), introduced the following change:

- Change of Principal Investigator in Hospital Valme (Spain).

Eligibility

Inclusion criteria

1. Patients 18 to 50 years of age, inclusive.
2. Patients diagnosed with multiple sclerosis, according to the revised McDonald criteria of 2010,[20] with a relapsing–remitting course, and at least nine T2 lesions consistent with the disease located in the brain.
3. Patients with disease duration of between 1 and 5 years from the date of onset of first symptoms.
4. Patients who had at least two relapses in the past 2 years. This criterion was applicable only for the Spanish population.
5. Patients prescribed with fingolimod who met Pharmaceutical Benefits Scheme (PBS) eligibility criteria for fingolimod. This criterion was applicable only for the Australian population.
6. Patients with an EDSS score[22] between 0 and 3.5, inclusive.
7. Patients:
    a. treatment-naïve: patients who had never been treated with a DMT, or
    b. previously treated with first-line DMT: patients who had been treated with a first-line DMT continuously for at least a 1-year period (interferon beta-1a [intramuscular or subcutaneous], interferon beta-1b or glatiramer acetate).
8. Patients who granted their written consent after the nature and purpose of the research had been explained clearly to them (informed consent in writing).

Exclusion criteria

1. Patients with clinical symptoms of MS different from relapsing–remitting MS.
2. Patients with a history of chronic immune system disease other than MS, such as documented acquired immunodeficiency syndrome (AIDS).

3. Patients with a history of treated or untreated malignant disease of any organ system (except localized basal cell carcinoma of the skin) in the last 5 years, regardless of whether evidence of local relapse or metastasis exists.
4. Patients with uncontrolled diabetes mellitus (HbA1c > 7%).
5. Patients with a suspected diagnosis of macular oedema during the screening visit. (Patients with a history of macular oedema were allowed to take part in the study provided that they did not present with macular oedema at the screening visit.) At Visit -1 (screening), if macular oedema (e.g. loss of visual acuity) was suspected, the patient was classified as a screening failure and not included.
6. Patients with active systemic bacterial, viral or fungal infections, with documented diagnosis of AIDS, hepatitis B or hepatitis C or who tested positive for IgG antibodies against HIV, for hepatitis surface antigen B or for hepatitis C antibodies.
7. Patients who tested negative for IgG antibodies against the varicella-zoster, rubella or measles viruses at the screening visit. Patients could be vaccinated and, once it was established that they had IgG antibodies, could be included 1 month after vaccination.
8. Patients who received live or live attenuated vaccines in the 2 months prior to the baseline visit[33] for varicella-zoster virus, rubella and measles refer above, could be included 1 month after vaccination
9. Patients who received total lymphoid irradiation or bone marrow transplantation in the 2 months prior to the baseline visit.
10. Patients who had received treatment with:
    a. fingolimod at any time (e.g. participation in a fingolimod clinical trial)
    b. immunosuppressant drugs such as azathioprine or methotrexate at any time
    c. immunoglobulins in the past 6 months (prior to V-1)
    d. monoclonal antibodies, including natalizumab, at any time
    e. cladribine, cyclophosphamide or mitoxantrone at any time.

11. Patients with any unstable medical condition as assessed by the primary treating physician in each centre, for example patients with cardiac risk factors (any degree of atrioventricular block, branch block conduction abnormalities or intraventricular conduction block, or patients who have had severe cardiac syncope) or with a slow (bradycardia) or irregular heartbeat.
12. Patients who had taken part in a clinical trial within the last 3 months.
13. Women of childbearing potential who were planning to become pregnant, were pregnant and/or breastfeeding, or who did not wish to use effective contraception, for example hormonal contraceptives (implantation, patches, oral) or double-barrier methods (any double combination of: intrauterine device, male or female condoms with spermicidal gel, diaphragm, contraceptive sponge, cervical cap).
14. Patients with severe hepatic impairment (Child-Pugh class C).
15. Patients who presented the following medical conditions:

a. second degree Mobitz type II or higher atrioventricular block, sick-sinus syndrome, or sino-atrial heart block
b. prolongation of the QT interval [QTc >470 msec (females) or >450 msec (males)].
c. history of bradycardia or recurrent syncope, ischaemic heart disease, cerebrovascular disease, history of myocardial infarction, congestive heart failure, history of cardiac arrest, uncontrolled hypertension or severe untreated sleep apnoea period.

16. Patients receiving concurrent therapy with anti-arrhythmic or treatments which could decrease heart rate:

a. arrhythmias treatment with class Ia (e.g. quinidine, procainamide) or class III anti-arrhythmic drugs (e.g. amiodarone, sotalol)
b. betablockers
c. heart rate-lowering calcium channel blockers (such as verapamil, diltiazem or ivabradine)

d. other substances which could decrease heart rate (e.g. digoxin, anticholinesterase agents or pilocarpine).

Recruitment

EARLIMS was designed at the time fingolimod was approved as a first-line therapy in the USA (September 2010) and Australia (January 2011). However, the label in Europe was approved in March 2011 with a second-line indication, which affected recruitment plans. Additionally, where indicated first line, fingolimod is often used in clinical practice as a first DMT-switch option. Of the 460 patients screened in EARLIMS, 113 did not meet the inclusion criteria, mainly owing to not fulfilling criteria of disease stage, severity or duration, and lack of immunity for varicella-zoster, rubella and measles. Together, these factors impacted the ability of study sites to recruit. Despite efforts to motivate recruitment and a one-year extension, the planned sample size was not attained and a further extension to recruitment was not possible. Based on the actual sample size, the study had 28% power to detect a difference of −0.1 between a Group 1 mean of 0.214 and a Group 2 mean of 0.301, assuming that the common standard deviation was 0.5594, and with a significance level (alpha) of 0.05 using a two-sided two-sample t-test.