FINAL STUDY REPORT

An exploratory phase IIa study to evaluate the safety and immunological effects of intravenous interferon Beta-1a (IFN Beta-1a, Rebif® New Formulation) therapy in the induction of tolerance to IFN Beta in MS patients with neutralising antibodies (NAbs) to subcutaneous IFN Beta-1a (Rebif® or Avonex®)

Short title: Tolerance induction with intravenous IFN Beta-1a

Study Description: Proof of concept study to assess the safety and immunological effects of high dose intravenous IFN Beta-1a human serum albumin-free formulation (Rebif® New Formulation) after transient, peripheral leukocyte depletion with Mitoxantrone with the objective to tolerize patients with NAbs to IFN Beta.

Sponsor: Queen Mary University of London

Sponsor Reference: 6114

EudraCT Number: 2008-000256-26

REC Reference: 08/H0305/64

First Patient First Visit: 22 May 2012

Last Patient Last Visit: 03 Sep 2013

Chief Investigator:

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Report Date: 11 Feb 2015

The study was conducted in accordance with the Declaration of Helsinki, consistent with International Conference on Harmonisation - Good Clinical Practice (ICH-GCP).

Abbreviations:

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TIW	Three times per week
BUN	Blood urea nitrogen
HCG	Human chorionic gonadotropin
Hgb	Haemoglobin
ICH	International Congress of Harmonisation
IFNβ	Interferon beta
SAE	Serious adverse event
SI	Système International
SOP	Standard Operating Procedure
SRB	Safety review board
s.c.	sub-cutaneous
i.m.	intra-muscular
WHO	World Health Organization
Nabs	Neutralising antibodies

Contents

Ethical Considerations

Ethical approval

This study was given favourable ethical approval by Cambridgeshire 4 REC on 16th February 2009. Further amendments were made which also gained ethical approval from the some REC Institution. Appendix 1 provides a tracker for the study amendments and their approval dates.

Conflicts of interest

The NAB Anergy study was funded by Merck-Serono. Prof Giovannoni has received grants and personal funding from Roche, Gilead, Novartis, BMS, BI, Chughai, Idenix and GSK. The study team can confirm that the funders played no role in the conduct of the study nor the analysis of the results.

Patient consent and enrolment

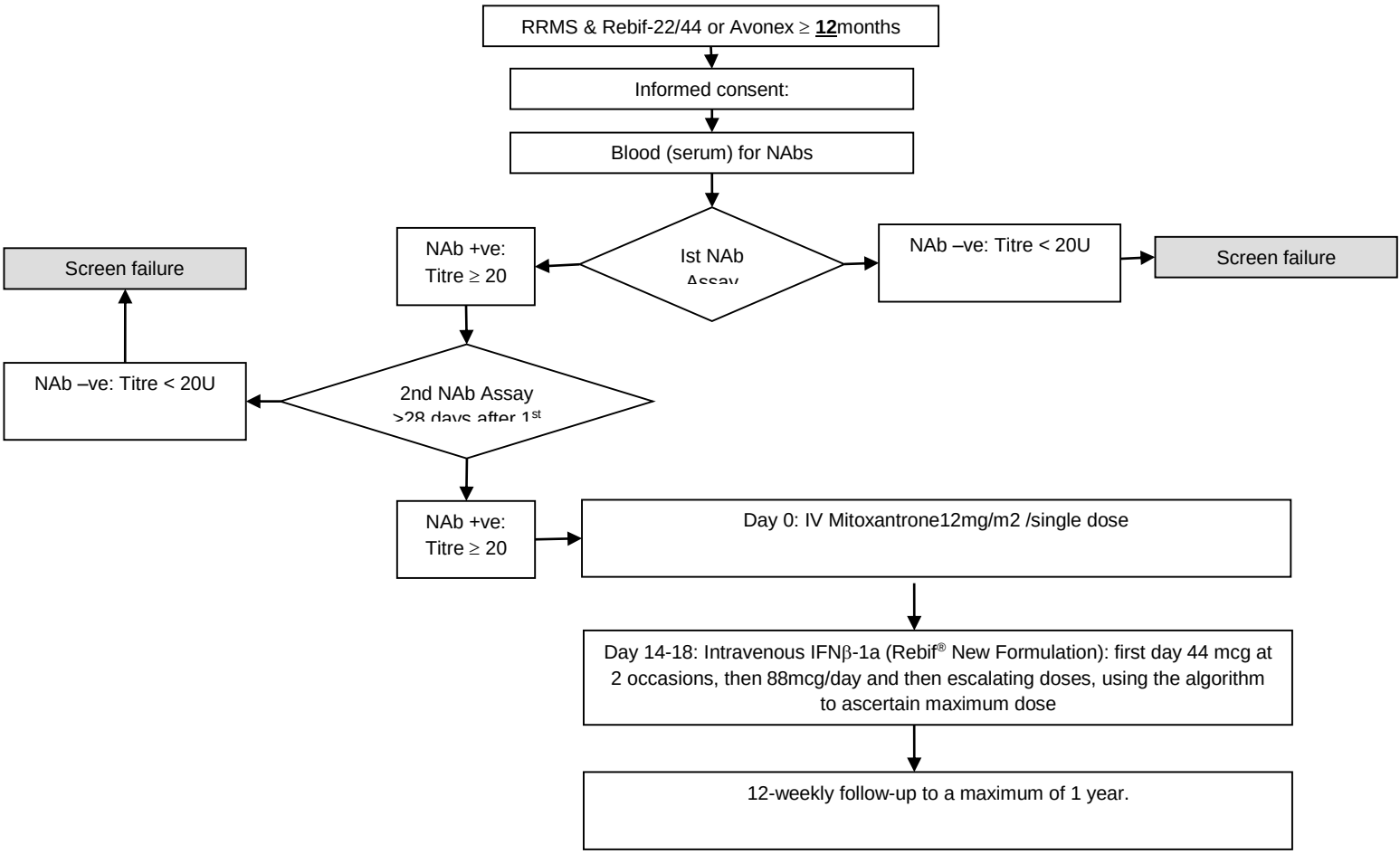
One patient was consented to the study. The patient gave written informed consent before any study specific assessments were performed. Investigators completed Eligibility Screening Criteria with the patient, documenting her eligibility with regard to the inclusion and exclusion criteria for the study.

Investigational Plan

Study design

This was an open label, UK study involving 1site.

Study Design Diagram



Patient selection

Male and female subjects with MS, aged between 18 and 65 years of age (inclusive), who have been on IFNβ-1a (Rebif-22/44 or Avonex) for at least 12 months and have at least one significant relapse in the last 12 months and are considering switching therapy.

They will be screened for the presence of NAbs using a bioassay.

Subjects with a positive NAb titre of 20U will then be invited to volunteer to continue in the study. The study will be a single-centre open-label study to assess the therapeutic efficacy of intravenous Rebif New Formulation after transient peripheral leukocyte depletion.

Leukocyte depletion will be induced by a single course of intravenous Mitoxantrone (12mg/m2). Intravenous IFNβ-1a will be administered approximately during leukocyte proliferation day 10 to 15 after the administration of Mitoxantrone.

Inclusion criteria

Confirmed diagnosis of MS according to the McDonald criteria Appendix.

Current treatment with Rebif® (22 or 44) s.c. TIW or Avonex® i.m. once per week for at least 12 months

At least one relapse in the last 12 months

Two consecutive positive NAb titers of 20NU at least 4 weeks apart.

Age over 18 years and less than 65 years.

Expanded Disability Status Scale (EDSS) score not to exceed 6.5.

Women of childbearing potential and men must agree to practice adequate contraception methods, defined as barrier methods with spermicide, surgical sterilisation of self or male partner, combined oral contraceptives or intrauterine device/system during the study. All female subjects who are not post-menopausal or surgically sterile must have a negative blood pregnancy test at screening.

Must give written informed consent and authorize the release and use of protected health information, as required by local law.

Able and willing to undergo blood sampling at regular intervals as defined by the protocol.

Able to comply with study requirements.

Exclusion criteria

Treatment with other immunosuppressive, immunomodulatory, or experimental treatments within the last 6 months of enrollment in the study, but excluding pulsed intravenous or oral steroids for treatment of MS relapse relapse or IFNβ.

History of WHO grade 3 or 4 liver toxicity with IFNβ-1a Patients presenting a severe or unstable disorder: poorly controlled diabetes, arterial hypertension, severe cardiac insufficiency, unstable ischemic heart disease, abnormal liver function tests (>2.5 times ULN) and abnormal complete blood count (in particular leukopenia and thrombocytopenia 1.5 LLN), or any medical condition which, in the opinion of the chief investigator, would pose additional risk in administering IFNβ-1 to the patient.

Presence of chronic or recurrent infection or human immunodeficiency virus.

Exposure to any other investigational drug within 30 days of enrollment in the study.

History of malignancy unless an exception is granted by the Chief Investigator.

History of drug or alcohol abuse within 6 months prior to enrollment into the study.

Pregnancy or breast feeding.

Left Ventricular Ejection Fraction (LVEF) < 50% on echocardiogram or an abnormal 12-lead ECG

Investigational Medicinal Product (IMP)

Rebif® New Formulation EU/1/98/063/004, EU/1/98/063/005, EU/1/98/063/006

Mitoxantrone PL 04515/0127

Efficacy endpoints

To assess the therapeutic safety and efficacy of intravenous RNF after transient peripheral leukocyte depletion.

The effect of this strategy was only analysed for the one patient for NAb titre at 3, 6, 9 and 12 months post intravenous IFN -1a relative to baseline.

The T-cell proliferative response to rhuIFN -1a compared to baseline, change in T-and B-cell cytokine production in response to rhu-IFN -1a, as assessed using Elispot assays, intracellular cytokine staining, protein and mRNA levels, every 3 months post intravenous IFN-1a relative to baseline and the antigen-specific response will be compared to the changes in control

antigen (tetanus toxoid) were not tested as only one patient was enrolled and completed and no significant value would be added with these analysis for one patient only.

Safety assessments

AEs were monitored throughout the trial. Laboratory (haematology and biochemistry) tests, and physical examinations were performed at screening and thereafter at regular intervals throughout the trial.

SAE reported for Grade-4 laboratory toxicity related to high dose Interferon-beta injection

Expected: Expected for both IMPs

Outcome: Resolved

Dates of treatment: 24/09/2012-27/09/2012

Date of onset: 28/09/2012

Route of admin.: IV

Dosage form: Rebif NF - 132mcg, IV, OD

Daily dose: Rebif NF - 132mcg, IV, OD

IMP Causality: Related to Rebif New Formulation, Unrelated to Mitoxantrone

Sex: F

Date of Birth: 05/09/1975

Country: UK

Body System: Blood and Lymphatic System

Subject ID: 00001

Case Ref No.: AAA01

Statistical analysis

Planned descriptive statistics in the different dose cohorts were not done as only one patient was enrolled and finished the study.

Patients

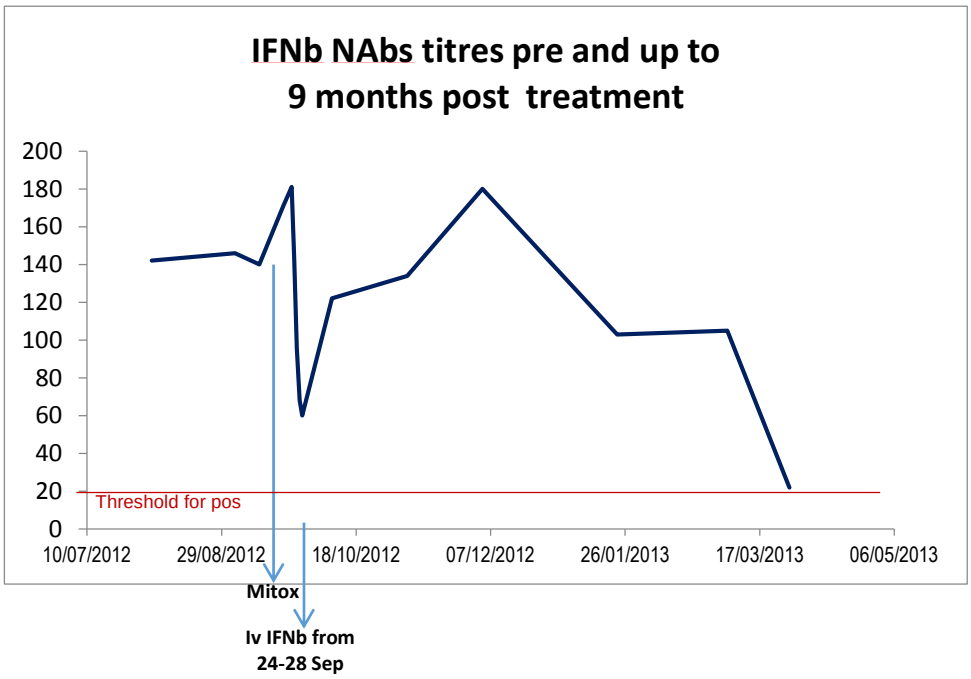
One patient was recruited to the study, signed the Informed Consent Form and finished the trial.

Protocol deviations

1. Late submission of DSUR for period up to November 2013 was submitted late to the MHRA. The DSUR should have been sent within 60 days of 24 November, but was sent on 9 April 2013. This late reporting was acknowledged by MHRA on 9 July 2013.

Efficacy

We assessed the patient's Nabs as it mattered for patient's further MS treatment. The NABs were reduced for this participant. This was a tertiary end-point. The primary and secondary end point efficacy measurements were not assessed as just one patient finished the study.



Safety

There was a SAE as for Grade 4 laboratory results that caused the last dose of interferon not to be injected, as per protocol. There were no other relevant safety issues with the trial's only participant.

Discussion

Early termination with only one patient recruited and finished out of the 15 (5 for 3 different doses) patients planned meant we could not evaluate the efficacy of the IMP (mitoxantrone followed by high dose iv interferon-beta).

The only person who finished the treatment had a positive result at 12 months: the NAbs titres were reduced and could continue to use Interferon-beta as a disease modifying drug for MS. We cannot extrapolate for other individuals.

As discussed with the DSMB meeting, the overall risk benefit assessment was deemed too high to continue the trial. At the time of End of Trial there were several options available for people with MS who were on Interferon-beta and developed NAbs: either teriflunomide (an oral drug with similar efficacy) or escalation of treatment to natalizumab or fingolimod. The use of mitoxantrone in our trial possibly precludes the future use of drugs such as natalizumab as it significantly increases the side-effect profile.

Appendix 1 – Amendment Tracker

AMENDMENT NAME	DATE APPROVED BY ETHICS	DATE APPROVED BY MHRA	DATE APPROVED BY R&D	Documents Included
Initial Submission	Invalid letter 25 March 2008	Invalid letter 10 October 2008	N/A	• Protocol V1.0 • PIS V1.0 • ICF V1.0 • GP letter V1.0
Initial Approved Submission	16 February 2009	24 November 2008	20 February 2009	• Protocol V2.0 • GP letter V2.0 • ICF V1.0 • PIS V1.0
Substantial Amendment 1	Invalid letter 23 March 2010	Amendment re-sent	N/A	• Protocol V3.0 • PIS V3.0
Substantial Amendment 2	26 August 2010	26 March 2010	08 April 2011	• Protocol V3.0 • PIS V4.0 • ICF for donation and storage of tissue samples for future medical research V2.0
Substantial Amendment 3	11 November 2013	04 December 2013	13 November 2013	• Protocol V5.0
Non-Sub Amendment 1	11 September 2012		12 September 2012	• Protocol V4.0 • Guidelines for mitoxantrone administration in MS
Non-Sub Amendment 2	29 June 2012		15 April 2013	• PIS V5.0 • ICF V2.0

5.11. Visit Summary

	Visits 1 – 3				Visit 5* – 10*				
Study Procedures	**Visit 1 (Day -60)**	**Visit 2 (Day -30)**	**Visit 3 Day 0**	**Visit 4 (Day 11)**	**Visit 5* (Day 14)**	**Visit 6* (Day 15)**	**Visit 7* (Day 16)**	**Visit 8* (Day 17)**	**Visit 9* (Day 18)**
	(Screening)	*(1 month after Visit 1)*	*(within **4** weeks of Visit 2)*		*(Day 1 of infusion)*	*(Day 2 of infusion)*	*(Day 3 of infusion)*	*(Day 4 of infusion)*	*(Day 5 of infusion)*
Meeting with study doctor/Review of study procedures/ MS history/Medical history/Medications	**X**		**X**		**X**	**X**	**X**	**X**	**X**
Consent	**X**								
Changes to MS history/Medical history/Medications	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**
Blood tests; Neutralising antibodies; full blood count; liver function tests	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Neurological Exam - EDSS	**X**								

Ultrasound of heart (Echocardiogram) & ECG		**X**		**X[1]**					
Blood pregnancy test (Females only)		**X**							
Urine Pregnancy test (Females only)					**X**				
IV Mitoxantrone			**X**						
IV Rebif® New Formulation					**X**	**X**	**X**	**X**	**X**
Adverse Event recording			**X**		**X**	**X**	**X**	**X**	**X**
*NB: Visits may be delayed by one week if blood results are abnormal (Please refer to section 5.12.5 Visit 4-8: Day 14-18 (IV Tolerance Protocol)									

5.11. Visit Summary (Continued)

	Visit 10 – 12			Visits 13 – 15		
Study Procedures	**Visit 10/Month 1** **Day 25 (+/- 3 days)**	**Visit 11/Month 2**	**Visit 12/Month 3**	**Visit 13/Month 6**	**Visit 14/Month 9**	**Visit 15/Month 12** **Final**
	1 week post infusion (+/- 3 days)	*(Monthly visits after Visit 10)*		*(3 Monthly Visits after Visit 12)*		
Meeting with study doctor/Review of study procedures/ MS history/Medical history/Medications	**X**	**X**	**X**	**X**	**X**	**X**
Changes to MS history/Medical history/Medications	**X**	**X**	**X**	**X**	**X**	**X**
Blood tests; Neutralising antibodies; full blood count; liver function tests	**X**	**X**	**X**	**X**	**X**	**X**
Neurological Exam						**X**

- EDSS						
Urine Pregnancy test (Females only)		**X**		**X**	**X**	
IV Mitoxantrone						
Adverse Event recording	**X**	**X**	**X**	**X**	**X**	**X**

[1] ECG only at Visit 4