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# *Assessment of bone marrow-derived Cellular Therapy in progressive Multiple Sclerosis*

## Statistical Analysis Plan

| | NAME | TITLE | SIGNATURE | DATE |
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## Abbreviations

| | |
|---|---|
| ACTIMUS | Assessment of bone marrow-derived cellular therapy in progressive multiple sclerosis |
| BAEP | Brainstem auditory evoked potential |
| CCT | Central motor conduction time |
| EDSS | Expanded disability status scale |
| GEP | Global evoked potential |
| ITT | Intention to Treat |
| LL | Lower limb |
| MRI | Magnetic resonance imaging |
| MSIS-29 | Multiple sclerosis impact scale |
| MSFC | Multiple sclerosis functional composite |
| OCT | Optical coherence tomography |
| PPMS | Primary progressive multiple sclerosis |
| SEP | Sensory evoked potential |
| SPMS | Secondary progressive multiple sclerosis |
| UL | Upper limb |
| VEP | Visual evoked potential |

**ACTIMUS STUDY GROUP**

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Denise Owen - data acquisition
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Peter Walsh - trial design, acquisition and interpretation of neurophysiology outcome measures

## 1. INTRODUCTION & PURPOSE

This document details the rules proposed and the presentation that will be followed, as closely as possible, when analysing and reporting the main results from the **ACTIMUS** study.

The purpose of the plan is to:

1. Ensure that the analysis is appropriate for the aims of the trial, reflects good statistical practice, and that interpretation of a priori and post hoc analyses respectively is appropriate.
2. Explain in detail how the data will be handled and analyzed to enable others to perform the analysis in the event of sickness or other absence

Additional exploratory or auxiliary analyses of data not specified in the protocol are permitted but fall outside the scope of this analysis plan (although such analyses would be expected to follow Good Statistical Practice).

The analysis strategy will be made available if required by journal editors or referees when the main papers are submitted for publication. Additional analyses suggested by reviewers or editors will, if considered appropriate, be performed in accordance with the Analysis Plan, but if reported the source of such a post-hoc analysis will be declared.

Subsequent versions of the statistical analysis plan will include a table of changes made since the previous version. When the plan cannot be followed in the primary effectiveness analysis, infringements of the plan will be detailed with reasons in the report of primary results.

# 2. SYNOPSIS OF STUDY DESIGN AND PROCEDURES

## 2.1 Trial objectives and aims

The following study synopsis has been written with the sole purpose of informing this statistical analysis plan. For details of the study to inform any other purpose, the current version of the protocol must be consulted (Rice et al 2015).

### 2.1.1 Primary trial objective

To determine the efficacy of intravenous infusion of autologous bone marrow cells in patients with progressive multiple sclerosis.

### 2.1.2 Secondary trial objective

To collect additional safety data regarding the collection and intravenous infusion of bone marrow cells in those with multiple sclerosis.

## 2.2 Trial design and configuration

ACTIMUS is a double blind, randomised, placebo-controlled parallel group trial. Each participant will receive two infusions, one of marrow (the intervention) and one of blood (the comparison), the order or these infusions being randomly allocated. Multimodal evoked potentials will be examined at 0, 6, 12, 18 and 24 months.

There are potential differences in the efficacy of immediate and delayed marrow infusions, hence the data will not be analysed as if from a cross-over trial.

## 2.3 Trial centres

ACTIMUS is a single centre study based at Southmead Hospital, Bristol.

## 2.4 Eligibility criteria

### 2.4.1 Inclusion criteria

18 to 65 years of age

Diagnosis of clinically definite multiple sclerosis as defined by the McDonald criteria

Disease severity EDSS 4-6

Disease duration >5 years

Disease progression (not attributable to relapse) in the year prior to entry

Signed, written informed consent

Willing and able to comply with study visits according to the protocol for the full study period

### 2.4.2 Exclusion criteria

Pregnancy, breast feeding or lactation

History of autologous/allogeneic bone marrow transplantation or peripheral blood stem cell transplant

Bone marrow insufficiency

History of lymphoproliferative disease or previous total lymphoid irradiation

Immune deficiency

History of current or recent (<5 years) malignancy

Chronic or frequent drug-resistant bacterial infections or presence of active infection requiring antimicrobial treatment

Frequent and/or serious viral infection

Systemic or invasive fungal disease within 2 years of entry to study

Significant renal, hepatic cardiac or respiratory dysfunction

Contraindication to anaesthesia

Bleeding or clotting diathesis

Current or recent (within preceding 12 months) immunomodulatory therapy other than corticosteroid therapy

Treatment with corticosteroids within the preceding 3 months

Significant relapse within the previous 6 months

Predominantly relapsing-remitting disease over the preceding 12 months

Radiation exposure in the past year other than chest/dental x-rays

Previous claustrophobia

The presence of any implanted metal or other contraindications to MRI

Participation in another experimental study or treatment within the past 24 months

## 2.5 Description of interventions

See Rice et al (2010) for a report of feasibility work on the interventions being evaluated in the present study.

### 2.5.1 Venesection

Venesection of approximately 500 mL will be performed at entry and at one year.

### 2.5.2 Bone marrow harvest and infusion

Approximately 500-600 mL marrow will be collected together with bone marrow trephine at entry.

### 2.5.3 Study interventions

An infusion of either blood or marrow will be performed, according to random allocation, at the start of the first twelve-month study period on the day of the bone marrow harvest.

One year later, infusion of blood or thawed marrow will be performed (which ever the participant did not receive at the time of harvest.

### 2.6 Randomisation procedures

Randomisation will be carried out centrally, allocations only being released once the new study participant is logged into the system, thus ensuring allocation concealment. Each participant will be randomly allocated to marrow infusion at entry and blood infusion after twelve months, or blood infusion at entry and marrow infusion at twelve months. Randomisation will be stratified by disease type (PPMS versus SPMS) using a permuted block approach to ensure, for each disease type, approximately equal numbers of participants allocated to immediate and delayed marrow infusion.

### 2.7 Sample size and justification

We will randomly allocate 40 patients to each treatment order group, 30 with SPMS and ten with PPMS. This will allow an overall standardised difference of 0.73 in global evoked potential between the groups to be detected with 80% power at the 5% significance level, assuming loss to follow-up of 25%.

### 2.8 Blinding

During infusion the trial product (blood or marrow) will be shielded from the participant using covered giving sets and obscuring the cannula site from the participant. Outcome assessors will not have access to information regarding order of the two infusions.

### 2.9 Outcome measures

#### 2.9.1 Primary outcome

The change in global evoked potential score during the first 12-month study period will be the primary outcome measure.

Evoked potential abnormalities will be quantified according to a four-point graded ordinal score modified from Leocani et al (2006): 0=Normal; 1=Increased latency; 2=Increased latency and abnormal amplitude; 3=Absent. The global evoked potential score will be calculated as the sum of left and right brainstem auditory evoked potential and visual evoked potential scores (0-12) and left and right upper and lower sensory evoked potentials (0-12) and central motor conduction times (0-12). Further details are presented in the following section.

### 2.9.2 Evoked potential measures

The global score is the sum of abnormality scores on the following measures, range 0-36 with high scores indicating greater impairment (Leocani *et al*, 2006):

*Brainstem Auditory Evoked Potential (BAEP), left ear and right ear each scored as:*

**0** Normal latency

**1** Latency 6.4 ms or greater

**2** Not used

**3** No response

*Visual Evoked Potential (VEP), left eye and right eye each scored as:*

**0** Normal latency and amplitude

**1** Latency 108 ms or greater

**2** Latency 108 ms or greater plus amplitude 3 or less

**3** No response

*Median Sensory Evoked Potential (SEP-UL), left hand and right hand each scored as:*

**0** Normal latency and amplitude

**1** Latency 23 ms or greater

**2** Latency 23 ms or greater plus amplitude 0.8 or less

**3** No response

*Tibial Sensory Evoked Potential (SEP-LL), left leg and right leg each scored as:*

**0** Normal latency and amplitude

**1** Latency 43.9 ms or greater

**2** Latency 43.9 ms or greater plus amplitude 0.5 or less

**3** No response

*Upper limb Central motor Conduction Time (CCT-UL), left & right arm each scored as:*

**0** Normal latency

**1** Latency 7.2 ms or greater

**2** Not used

**3** No response

*Lower limb Central motor Conduction Time (CCT-LL), left & right leg each scored as:*

**0** Normal latency

**1** Latency 14.9 ms or greater

**2** Not used

**3** No response

### 2.9.3 Secondary outcomes

Multimodal evoked potentials will be examined at 0, 6, 12, 18 and 24 months. The global score will also be presented for these additional time points.

Clinical outcomes will be assessed at the start of each study period, and at 6 weeks, 6 months and 12 months during each study period. Measures will include the Expanded Disability Status Scale (EDSS; Kurtzke, 1983), the Multiple Sclerosis Functional Composite (MSFC; Fischer, 1999), and the Multiple Sclerosis Impact Scale (MSIS-29; Hobart, 2001).

Participants will undergo cranial and spinal MRI at enrolment, and at the end of each twelve-month study period. Measures of the following will be taken: lesion load, atrophy measures of the brain and of cross-sectional area of the spinal cord, changes in mean diffusivity.

Participants will undergo optical coherence tomography (OCT) which will give measures of the following at enrolment, and the end of each of the two study periods: macular volume and thickness of the retinal cell layer.

Adverse events will be recorded, these being any unfavourable and unintended sign, symptom or illness that develops or worsens during the period of the study. Adverse events include unwanted side effects, toxicity or sensitivity reactions, abnormal laboratory results, and injury or recurrent illness.

If an adverse event results in death, is life threatening, requires hospitalisation or prolongation of an ongoing hospitalisation, results in persistant or significant disability or incapacity, or is considered by the investigator to be an important medical event, then it will be reported as a serious adverse event. Hospital admissions for a procedure planned before entry into the study will not be recorded as a serious adverse event.

## 2.10 Interim analysis

The occurrence of serious adverse events will be reviewed by the study management group as they occur. No interim analyses of the efficacy outcomes are planned.

# 3. GENERAL ANALYSIS CONSIDERATIONS

## 3.1 Analysis populations

The primary effectiveness analysis will include data from the **"full analysis set"**; all eligible (see section 5.1) randomised participants **in the groups to which they were allocated** (i.e. according to the intention-to-treat principle) and who provide the primary outcome measure at the end of the first twelve-month study period.

The **"per protocol set"** will include all randomised participants, **in their allocated groups**, who were able to complete their allocated intervention in the first study period and who provide the primary outcome measure at the end of the first twelve-month study period. This set will be used in a sensitivity per protocol analysis of the primary outcome measure.

The "**safety set**" will group participants according to receipt of bone marrow or blood infusions, or no infusion at the start of the current study period. This set will be used for the reporting of adverse events.

## 3.2 Derived variables

Results based on derived variables will be sense-checked by the chief investigator.

## 3.3 Procedures for missing data

Missing outcome data will not be imputed, all analyses will be based on observed data.

# 4. DESCRIPTION OF PARTICIPANT CHARACTERISTICS

## 4.1 Disposition

The flow of patients through the trial will be summarised in a CONSORT diagram that will include the eligibility, reasons for exclusion, numbers randomised to the two treatment groups, those undergoing their allocated interventions, losses to follow up and the numbers analysed (Figure 1).

## 4.2 Baseline characteristics

Summary statistics for patient characteristics, as determined at baseline, will be presented in the text separately for those subsequently allocated to immediate or delayed marrow infusion. Characteristics will include age, sex, and disease duration at recruitment.

## 5. ANALYSIS OF EFFICACY

### 5.1 Patients found to be ineligible post-randomisation

For patients found to be ineligible post-randomisation, the reason for ineligibility will be noted, and the patient's participation in the study terminated (Figure 1). As this is an early evaluation of the intervention effectiveness, such patients will not be included in the analysis.

### 5.2 Summary of primary and secondary outcomes

For the full analysis set, summary statistics for the primary outcome, evoked potential measures, adverse events, EDSS, MSFC, MSIS-29, MRI measures, and OCT measures, will be presented at each time-point for the immediate and delayed marrow infusion groups. Individual trajectories in global evoked potentials will be presented graphically (Figure 2).

### 5.3 Primary analysis

The null hypotheses addressed by the primary analysis are that, in truth there is no difference in the global evoked potential between the immediate and delayed marrow infusion groups at the end of the first study twelve-month period.

The effectiveness of bone marrow infusion will be quantified as a difference in mean global evoked potential at twelve months (marrow infusion mean – blood infusion mean), amongst patients in the full analysis set (intention-to-treat estimate). The effectiveness will be estimated separately for those with PPMS and SPMS; if the intervention effects suggest a benefit of the intervention for both groups of participants, an overall estimate of effectiveness will be presented.

The intervention effect will be estimated in a linear regression model with a participant's global evoked potential at the end of the first twelve-month period as the outcome measure ($y_i$) and covariates: treatment allocation ($x_{1i}$=1: immediate marrow infusion; $x_{1i}$=0: delayed marrow infusion) and global evoked potential at enrolment ($x_{2i}$). For an estimate of the overall effect a dummy variable will be included distinguishing patients with PPMS ($x_{3i}$=1) or SPMS ($x_{3i}$=0). A normal distribution is assumed for the residual errors: $e_i \sim N(0,\sigma_e)$. The coefficient for the treatment allocation covariate ($\beta_1$) is the intention to treat estimate of treatment effectiveness, comparing immediate with delayed marrow infusion. In statistical notation:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + e_i$$

The assumptions of normally distributed residuals and heteroscedacity will be assessed by examining residual plots. The difference in means will be presented with 95% confidence interval, and p-value (Table 1).

## 5.4 Secondary analyses

The following secondary analyses will be done on an intention to treat basis using the full analysis set. The above regression model will be adapted to secondary measures, separately for the end of the first and second study periods.

### 5.4.1 Secondary evoked potential measures

The primary analysis will be adapted to address null hypothesis that there is no difference in the global evoked potential between the immediate and delayed marrow infusion groups at the end of the second twelve-month period (Supplementary Table A). This analysis will be repeated for 12 and 24 month assessments of Brainstem Auditory Evoked Potential (BAEP), Visual Evoked Potential (VEP), Sensory Evoked Potential (SEP), and Central motor Conduction Time (CCT)(Supplementary Table A).

### 5.4.2 MSFC

This is a three-part quantitative assessment including a timed walk, nine-hole peg test and paced auditory serial addition test. Performance on these three measures is combined to give a z-score. The analysis will estimate the difference in mean scores at the end of each study period in turn (Table 2).

### 5.4.3 Patient-based MSIS-29

The MSIS-29 has physical and psychological subscales, each scored between 0 and 100 with high scores indicating a severe negative impact of multiple sclerosis. In the case of missing items, where 50% or more items have been responded to, a subscale score can be imputed based on the completed items. The analysis will estimate the difference in mean scores at the end of each study period in turn (Table 2).

### 5.4.4 Physician-based EDSS

The EDSS is scored from 0 (normal neurologic exam) to 10 (death due to multiple sclerosis). Scores will be presented (Supplementary Table 2), in addition to which the analysis of EDSS will follow the established practice of using time to first progression of at least one point from a baseline EDSS of 4.0, 4.5 or 5.0, or at least a 0.5 point increase from a baseline EDSS of 5.5 or higher (Leocani et al, 2006). The cumulative proportion of participants in the two allocated groups who have progressed at 6 weeks, 6 and 12 months will be presented, with the intervention effect at 12 months estimated as a crude odds ratio with 95% confidence interval and p-value (Table 3).

### 5.4.5 MRI measures

The following measures will be taken from MRI: lesion volume, total brain volume, cerebrospinal fluid volume, and spinal cord cross sectional areas at C2-3 and T9-10. From these measures estimates will be derived of lesion load (lesion volume/total brain volume), brain atrophy (total brain volume / intercranial volume) and cord atrophy (C2-3/T9-10, and C2-3/intercranial volume). The derived measures will be compared between allocated

groups at the end of each study period in turn (Table 4); the original measures will be presented in supplementary material (Supplementary Table 3).

#### 5.4.6 OCT measures

Macular volume and thickness of retinal cell layer will each be compared between allocated groups at the end of each study period in turn (Table 4).

### 5.5 Adverse events and serious adverse events

For the safety set, adverse events, and serious adverse events will be categorised and the number of patients with an event falling into a particular category presented according to the treatment received at the start of the corresponding study period (Supplementary Table D).

### 5.6 Subgroup analysis

Findings for the PPMS and SPMS participants will be stratified and presented in separate papers. An interaction test would have very low statistical power and will not be performed.

### 5.7 Sensitivity analysis

The primary analysis will be repeated with the per protocol set to give estimates of treatment efficacy (Table 1).

A modest number of participants with secondary progressive MS had either their first (n=6) or second (n=7) study periods extended due to their end of periods assessments being postponed during the COVID-19 outbreak. The effect of this will be explored in the following two ways:

- The primary analysis will be repeated with these participants excluded, with the findings reported in the text.
- One or more covariates will be added to the analysis model to accommodate any relationship between the primary outcome response and the time since the start of the study period.

### 5.8 Exploratory analysis

Exploratory analyses will be clearly delineated from the analysis of primary and secondary outcomes in any statistical reports. The exploratory analysis will include prognostic and predictive factor analysis of disease progression and multivariate analysis of response data.

## 6. FIGURES AND TABLES FOR JOURNAL PUBLICATION OF PRIMARY ANALYSIS

The results for participants with primary progressive MS and secondary progressive MS will be presented in a single paper. The following are the intended figures and tables for that paper.

**Table 1.** Intervention effects on the Global Evoked Potential score, primary intention-to-treat (ITT) and sensitivity per protocol estimates

| | **Early infusion Mean (sd) n** | **Late infusion Mean (sd) n** | **Difference (95% CI)** | **p-value** |
|---|---|---|---|---|
| **Primary ITT analysis** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| | | | | |
| **Per protocol (sensitivity)** | | | | |
| Enrolment | | | | |
| 12 months | | | | |

**Table 2.** Intervention effects on patient reported measures, intention-to-treat (ITT) estimates

| | Early infusion Mean (sd) n | Late infusion Mean (sd) n | Difference (95% CI) | p-value |
|---|---|---|---|---|
| **MSFC** | | | | |
| Enrolment | | | | |
| +6 weeks | | | | |
| 6 months | | | | |
| 12 months | | | | |
| +6 weeks | | | | |
| 18 months | | | | |
| 24 months | | | | |
| | | | | |
| **MSIS-29** | | | | |
| Enrolment | | | | |
| +6 weeks | | | | |
| 6 months | | | | |
| 12 months | | | | |
| +6 weeks | | | | |
| 18 months | | | | |
| 24 months | | | | |

**Table 3.** Intervention effects on the EDSS progression measure, intention-to-treat (ITT) estimates

| **Progressed by:** | Early infusion n/N (%) | Late infusion n/N (%) | Odds ratio (95% CI) | p-value |
|---|---|---|---|---|
| 6 weeks | | | | |
| 6 months | | | | |
| 12 months | | | | |

**Table 4.** Intervention effects on imaging measures, intention-to-treat (ITT) estimates

| | Early infusion Mean (sd) n | Late infusion Mean (sd) n | Difference (95% CI) | p-value |
|---|---|---|---|---|
| **Lesion load** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Brain atrophy** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Cord atrophy (C2-3/T9-10)** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Cord atrophy** | | | | |
| **(C2-3/intracranial volume)** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **OCT macular volume** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **OCT retinal layer thickness** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |

**Supplementary Table A.** Intervention effects on secondary evoked potential measures, intention-to-treat (ITT) estimates

| | Early infusion Mean (sd) n | Late infusion Mean (sd) n | Difference (95% CI) | p-value |
|---|---|---|---|---|
| **Global evoked potential (range 0-36)** | | | | |
| Enrolment | | | | |
| 6 months | | | | |
| 12 months | | | | |
| 18 months | | | | |
| 24 months | | | | |
| **Brainstem evoked potential (range 0-6)** | | | | |
| Enrolment | | | | |
| 6 months | | | | |
| 12 months | | | | |
| 18 months | | | | |
| 24 months | | | | |
| **Visual evoked potential (range 0-6)** | | | | |
| Enrolment | | | | |
| 6 months | | | | |
| 12 months | | | | |
| 18 months | | | | |
| 24 months | | | | |
| **Sensory evoked potential (range 0-12)** | | | | |
| Enrolment | | | | |
| 6 months | | | | |
| 12 months | | | | |
| 18 months | | | | |
| 24 months | | | | |
| **Central motor conduction time (range 0-12)** | | | | |
| Enrolment | | | | |
| 6 months | | | | |
| 12 months | | | | |
| 18 months | | | | |
| 24 months | | | | |

**Supplementary Table B.** Summary statistics for EDSS scores, intention-to-treat (ITT) estimates

| | Early infusion | Late infusion |
|---|---|---|
| | **Mean (sd) n** | **Mean (sd) n** |
| **EDSS** | | |
| Enrolment | | |
| +6 weeks | | |
| 6 months | | |
| 12 months | | |
| +6 weeks | | |
| 18 months | | |
| 24 months | | |

**Supplementary Table C.** Intervention effects on original imaging measurements, intention-to-treat (ITT) estimates

| | Early infusion Mean (sd) n | Late infusion Mean (sd) n | Difference (95% CI) | p-value |
|---|---|---|---|---|
| **Lesion volume** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Total brain volume** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Cerebrospinal fluid volume** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Cord cross-sectional area C2-3** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |
| **Cord cross-sectional area T9-10** | | | | |
| Enrolment | | | | |
| 12 months | | | | |
| 24 months | | | | |

**Supplementary Table D.** Key adverse events in those participants undergoing intervention as allocated to a study period. Counts are of patients (%), who may have experienced more than one complication of a given type. [Examples are given in this table, those adverse events that occur will be reported].

| | **Infusion period (n=)** | **Control period (n=)** | **No intervention (n=)** |
|---|---|---|---|
| Increased lower limb spasticity following bone marrow harvest | | | |
| Acute urinary retention following bone marrow harvest | | | |
| Temporary exacerbation following general anaesthesia | | | |
| Hypovolaemia or anaemia following blood and marrow harvest | | | |
| Hypersensitivity to marrow cryopreservative | | | |
| Exacerbation due to sepsis | | | |
| Assessment at or admission to hospital following fall | | | |
| … | | | |

**Figure 1:** CONSORT Flow chart

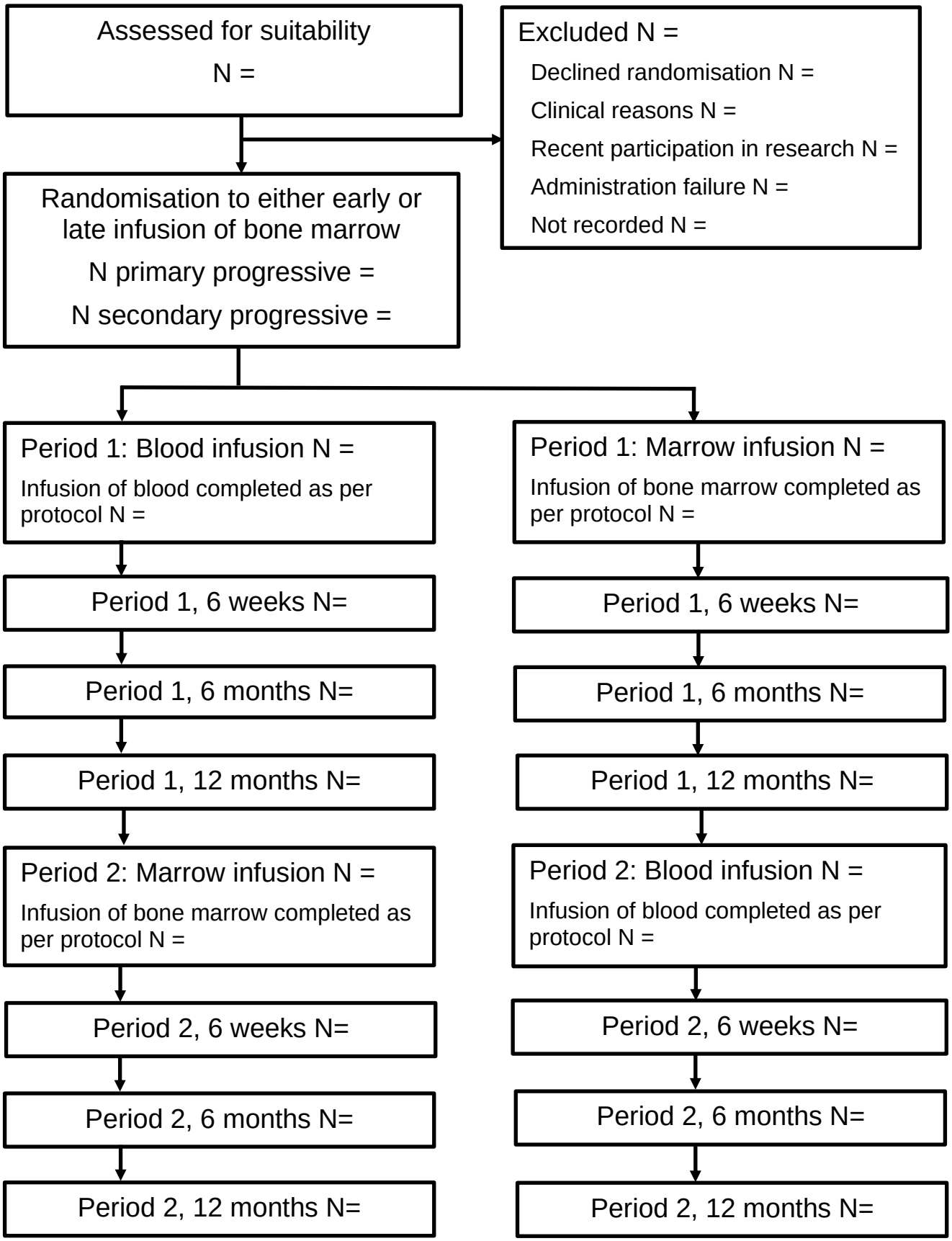
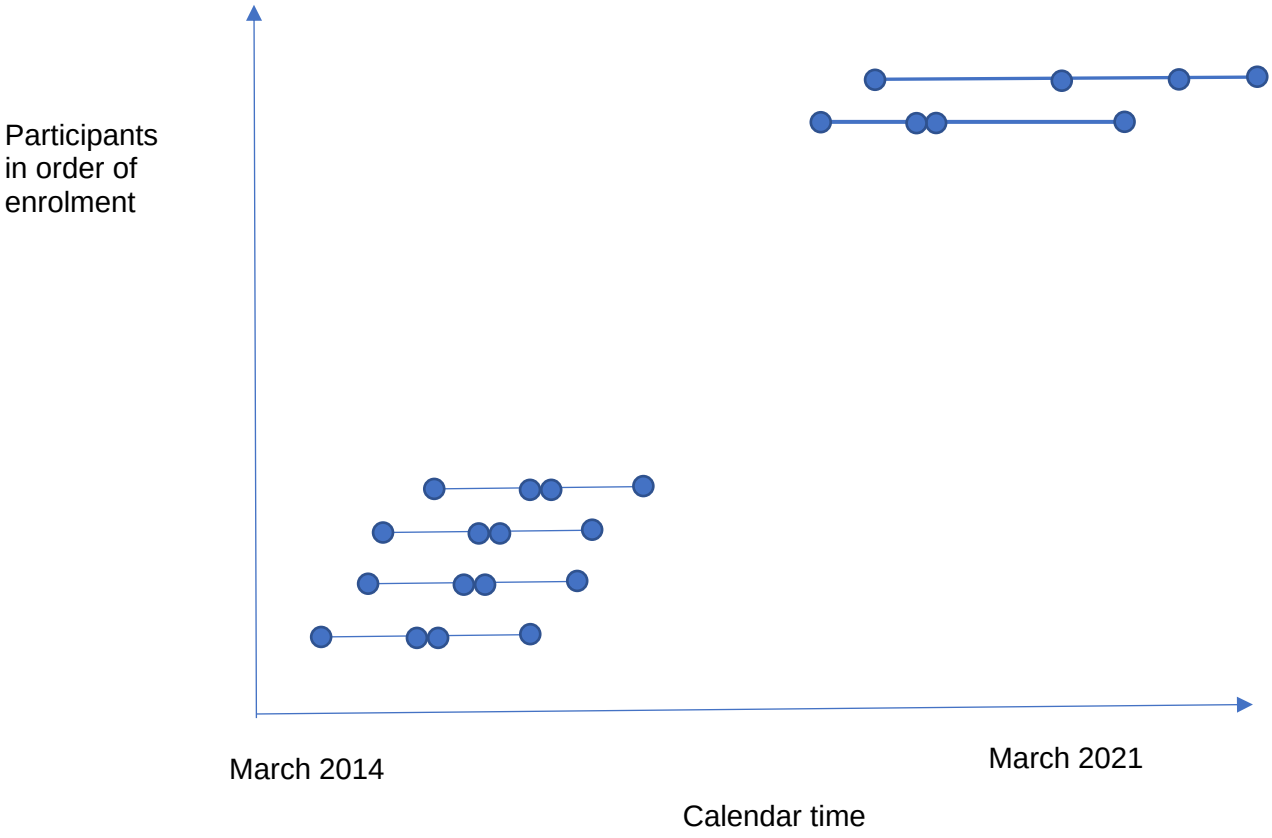


**Figure 2.** Individual trajectories in global evoked potentials for participants who received (a) early marrow infusion, (b) late marrow infusion.

**Supplementary Figure.** Participation timelines showing the impact of the COVID-19 pandemic. Each participant is represented by a horizontal series of connected time points, indicating the start and end of period 1, then the start and end of period 2. A bold connecting line indicates a participant with one or both 12 month assessments occurring more than 15 months after the start of the study period.
**[The following is an example and is not based on data.]**



## 7. REFERENCES

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