STREAMS

Stem cells in Rapidly Evolving Active Multiple Sclerosis

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Imperial College is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

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This protocol describes the STREAMS study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2 004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

2. TABLE OF CONTENTS

3. GLOSSARY OF ABBREVIATIONS

AE	Adverse event
ATMP	Advanced Therapy Medicinal Product
BM	Bone marrow
CMV	Cytomegalovirus
CNS	Central nervous system
CRF	Case report form
CRO	Clinical Research Organisation
CSF	Cerebrospinal fluid
ECG	Electrocardiogram
e-CRF	Electronic case report form
EDSS	Extended Disability Status Scale
FS	Functional systems
FSS	Functional subscores
GA	Glatiramer acetate
GCP	Good Clinical Practice
GEL	Gadolinium enhancing lesion
GP	General Practitioner
HIV	Human Immunodeficiency Virus
HTLV	Human T-cell Lymphotropic Virus
ICH-GCP	International Conference on Harmonisation on Good Clinical Practice
IFN-β	Interferon β
iPSP	Induced pluripotent stem cells
IV	Intravenous
LP	Lumbar puncture
MHRA	Medicines and Healthcare Regulatory Agency
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSC	Mesenchymal Stem/Stromal Cells
MSFC	Multiple Sclerosis Functional Composite
MSIS-29	Multiple Sclerosis Impact Scale-29
NB	Negative binomial
NHS	National Health Service
PASAT	Paced Auditory Serial Addition Test
PBMC	Peripheral blood mononuclear cell
PIS	Patient Information Sheet
PPMS	Primary progressive multiple sclerosis
R&D	Research and Development
RRMS	Relapsing remitting multiple sclerosis
SAE	Serious adverse event
SF-36	Short Form-36
SOP	Standard Operating Procedure
SPMS	Secondary progressive multiple sclerosis
SSA	Site Specific Assessment
SUSAR	Suspected unexpected serious adverse reaction
T25-FW	Timed 25-Foot Walk

KEYWORDS: Multiple Sclerosis, rapidly evolving, mesenchymal stem cells, bone marrow

4. STUDY SUMMARY

TITLE

Stem cells in Rapidly Evolving Active Multiple Sclerosis (STREAMS)

DESIGN

Randomised double-blind crossover study

AIMS

To describe the safety and efficacy of intravenously administered autologous MSCs in patients with Multiple sclerosis (MS).

OUTCOME MEASURES

- Primary outcome: To assess the safety of intravenous therapy with autologous MSCs in MS patients. The safety of MSCs infusion will be evaluated including frequency, timing and severity of any adverse events in both MSCs and placebo treatment groups. A statistical comparison of the number of adverse events between treatment groups will be carried out using a chi-square test.
- Co-Primary outcome: To assess the activity of autologous MSCs in MS patients. The total number of gadolinium-enhancing lesions (GEL) at MRI at weeks 4, 12 and 24 will be compared between treatment groups using a Negative Binomial (NB) regression analysis, adjusting for the number of GEL at baseline.
- Secondary outcomes: To gather preliminary information about the efficacy of the experimental treatment in terms of combined MRI activity, incidence of relapses and disability progression.

INCLUSION CRITERIA

Patients with clinically and radiologically active multiple sclerosis as defined by:

1. Diagnosis of MS:
   a. Relapsing remitting MS (RRMS): ≥1 moderate-severe relapse **and** ≥1 GEL in past 18 months **or** ≥1 moderate-severe relapse and ≥1 new T2 lesion in past 18 months.
   b. Secondary progressive MS (SPMS) with an increase of ≥1 EDSS point (if baseline EDSS ≤ 5.0) or 0.5 EDSS point (if baseline EDSS ≥5.5), in the previous 18 months **and** ≥1 GEL in past 18 months **or** ≥1 moderate-severe relapse and ≥1 new T2 lesion in past 18 months.
   c. Primary progressive MS (PPMS) patients with positive oligoclonal bands in the cerebrospinal fluid (CSF) and an increase of ≥1 EDSS point (if baseline EDSS ≤ 5.0) or 0.5 EDSS point (if baseline EDSS ≥5.5), or quantifiable, objective evidence of equivalent progression in the previous 18 months **and** ≥1 GEL in past 18 months or ≥1 new T2 lesion in past 18 months.
2. Age 18 to 50 years.
3. Disease duration 0 to 10 years from diagnosis (inclusive).
4. EDSS 2.0 to 6.5 at screening evaluation.
5. ≥1 GEL on MRI within 6 months prior to harvesting.
6. Adequate culture of subject's MSCs and their release for clinical use.

TREATMENT

At week 0 and at week 24 a single infusion of either *ex-vivo* expanded autologous MSCs or suspension media will be administered intravenously. MSCs will be given at 1-2 x 10^6 MSC/Kg recipient body weight as at baseline.

DURATION

4-year project with staggered recruitment of subjects.

5. INTRODUCTION

5.1 BACKGROUND

5.1.1 The burden of MS

Multiple sclerosis (MS) is an inflammatory demyelinating disease associated with neurological impairment and affects approximately 100,000 young adults in the UK alone. MS can cause severe neurological impairment and affects the quality of life of both the individual and their families. MS is also a major socio-economic burden for society; it costs the United Kingdom approximately £12,000 per patient per year in the early stages of the disease but this rises to nearly £60,000/year in the advanced stages [1].

5.1.2. The challenges of MS

Multiple sclerosis (MS) is a very complex disorder both clinically and pathologically [2]. It is a chronic inflammatory disease of the central nervous system (CNS), which ultimately leads to myelin damage and axonal loss. The cause is unknown. Aetiology is multifactorial, but the key pathogenic event appears to be an uncontrolled response of components of the immune system (T and B lymphocytes) to myelin proteins.

There are different forms of the disease. About 80% of the patients have a relapsing-remitting (RR) course that, in two-thirds of cases, will evolve into a secondary progressive (SP) course after about 10-15 years of the disease. About 20% of the patients have a progressive course from onset, called “primary progressive MS” (PPMS) [3]. It is believed that, in MS, inflammation and irreversible neurodegeneration coexist, with inflammation causing relapses and neurodegeneration being responsible for the irreversible disability that characterises most patients with chronic disease. Clinical severity of MS ranges from benign disease, in which the patient remains fully functional in all neurological systems (15 years after disease onset) to malignant MS which is characterised by an aggressive course resulting in accumulation of severe disability or death within a short time. The majority of patients suffer from a disease course of intermediate severity between these two extremes.

5.1.3. Current treatments for MS

No curative therapies exist for MS. As both the aetiology and pathogenesis underlying the disease are, as yet, unknown and due to the existing clinical heterogeneity in the MS population, treatments are suboptimal and have been challenging to develop.

Current immunomodulatory therapies for MS - including the use of interferon β (IFN-β), glatiramer acetate (GA) or mitoxantrone - are only partially effective, do not abolish inflammation and have only modest effects on disease progression. Natalizumab, a humanised antibody against the cellular adhesion molecule (α-4 integrin), prevents the adhesion of lymphocytes and monocytes to endothelial cells of the blood brain barrier and therefore interferes with the entry of inflammatory cells into the CNS. It is more efficacious than the aforementioned immunomodulatory drugs, reducing the relapse rate by 68% and progression of disability by 54% when compared to placebo. There is a 1:1000 risk of developing progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the CNS, in MS patients who have been treated with natalizumab for more than 2 years [4].

5.1.4. Development of new disease modifying treatments for MS

Recently, there have been significant developments in new treatment strategies for MS, including monoclonal antibodies for targeted immunomodulation (daclizumab, campath-1H, rituximab), limiting immune cell trafficking (e.g. fingolimod), or ‘resetting’ the immune system (through high-dose cyclophosphamide or haematopoietic stem cell transplantation) [5]. These therapies,

however, are at different stages of clinical development and evaluation of their safety and efficacy in treating MS requires further investigation.

5.1.5. Surrogate markers of disease in MS

Increasing evidence suggests that Magnetic Resonance Imaging (MRI) measures may offer sensitive, objective biomarkers of disease phenotype. MRI is the most widely utilised surrogate marker of disease activity in MS. Among MR metrics, the number of gadolinium-enhancing lesions (GEL) provides a measure of new inflammatory activity [6] and a large number of clinical trials have used gadolinium-enhanced lesions as the outcome measure. However, the relationship between such "conventional" MRI markers and the clinical evolution of disease is weak and MR metrics providing additional brain structural and metabolic information are expected to better predict clinical outcomes and capture important aspects of disease pathology [7].

5.2 RATIONALE FOR CURRENT STUDY

5.2.1. Mesenchymal stem/stromal cells (MSCs)

Mesenchymal stem/stromal cells (MSCs) are non-haematopoietic cells that reside in adult bone marrow and have the ability to self-renew and differentiate into tissues of mesodermal origin [8]. The relative ease by which MSCs can be expanded *ex-vivo* without significant manipulations has made them a potential candidate for use in regenerative medicine. They demonstrate extensive proliferative capacity *in vitro* and retain the ability to differentiate into several mesenchymal lineages, such as bone, cartilage, adipose tissue and muscle in lineage-specific cultures [9, 10]. Such multipotential capacity of MSCs appears to persist after transplantation - following intra-uterine injection of human MSCs into sheep, these cells engrafted and differentiated along multiple mesenchymal lineages (which was site-specific) and were still detectable for up to 13 months after transplantation [11]. *In vitro*, MSCs proliferate to spindle-shaped cells in confluent cultures. The cell yield after expansion varies with the age and condition of the donor and with the harvesting technique [12, 13]. In the absence of an agreed standardised marker, it is difficult to characterise MSCs so they are typically defined by a combination of phenotypic and functional characteristics. By flow cytometry, MSCs are negative for the haematopoietic markers CD14, CD34 and CD45. The cells remain positive for a number of adhesion molecules, including CD73, CD90, CD105 and CD166. The hallmark of MSCs is tri-lineage potential *in vitro*; i.e. the ability to differentiate into bone, cartilage and fat upon appropriate induction. MSCs give rise to the supportive stroma that constitutes the haematopoietic microenvironment and so sustain haematopoiesis *in vitro* by expression of a variety of haematopoietic cytokines (including IL-6, IL-11, leukemia inhibitory factor, stem cell factor and flt3/flk2 ligand) and growth factors but also via direct physical association [14, 15]. Human MSCs express human leukocyte antigen (HLA) class I and can be induced by interferon-gamma (IFNγ) to express HLA class II which should allow recognition by alloreactive T cells. However, this does not happen suggesting that MSCs escape detection by the immune system. They have immunomodulatory effects demonstrated in co-culture experiments in which human MSCs (even after being pre-cultured with IFN-γ) fail to induce proliferation of allogeneic lymphocytes, as reviewed in [16]. MSCs also escape lysis by cytotoxic lymphocytes (CTLs) despite expression of HLA class 1, the usual targets for CTL lysis, and by Killer-cell Immunoglobulin-like Receptors (KIR) ligand-mismatched Natural Killer cells [17]. When MSCs are present in mixed lymphocyte cultures (MLC), T-cell proliferation is suppressed [18]. The suppression is independent of HLA and occurs when added MSCs are autologous to the stimulatory or responder lymphocytes, or derived from a third party donor. Lymphocyte proliferation induced by mitogens is also markedly reduced in the presence of autologous or allogeneic MSCs [18]. MSCs which have differentiated into adipocytes, osteoblasts and chondrocytes retain the capacity to reduce lymphocyte proliferation in MLC [19]. In fact, differentiation, particularly along the osteogenic lineage, enhances their immunosuppressive effect.

5.2.2. Preclinical work on MSCs as relevant to multiple sclerosis

Uccelli and colleagues have extensively evaluated the effect of MSCs on experimental autoimmune encephalomyelitis (EAE), an experimental model of MS. They demonstrated that MSCs infused intravenously homed to lymphoid organs, ameliorated EAE and reduced infiltration of the CNS by T cells, B cells and macrophages [20]. More recently the same group demonstrated that MSCs migrate into the inflamed CNS and whilst they do not differentiate into neural cells, this is still associated with reduced demyelination and axonal loss [21]. These results suggest that MSCs exert their therapeutic activity on the inflamed CNS through the peripheral control of immune cells and a protective effect *in situ* [22]. In addition, MSCs could foster endogenous repair through the recruitment of local neural precursor cells leading to some degree of endogenous neurogenesis and remyelination [23]. Many other features are also likely to be relevant for exploitation of MSCs in MS and CNS related diseases. MSCs display a potent antioxidant effect in EAE affected mice [24] and this neuroprotective effect may result from the release of anti-apoptotic molecules [25] and of neurotrophins [26]. These results support the concept that MSCs are likely to foster CNS repair not only by acting as tolerogenic cells, but also as bioactive providers of trophic and anti-apoptotic factors leading to neuroprotection [27]. Autologous bone marrow-derived MSCs from MS patients exhibit the same properties as MSCs from healthy donors in terms of proliferation, phenotype, *in vitro* differentiation and immunosuppressive ability [28, 29].

5.2.3. Clinical experience of MSC therapy in humans

Safety: It has been estimated that at least 2000 patients in total have been treated with MSCs either within the clinical trial setting or as anecdotal cases. This includes a number of patients with stroke and MS with no reports of significant graft related complications such as induction of immunodeficiency and its attendant complications of opportunistic infections and malignancy. The procedure is simple, relatively cheap, potentially widely applicable and the autologous source is ethically acceptable to all patients. Recent studies have shown that MSCs from autoimmune patients display normal ability to support haematopoiesis [30], immunomodulatory activity [31] and surface and molecular phenotype [32], thus supporting the possibility of safely utilising autologous MSCs for transplantation purposes in these patients.

Clinical experience in Graft-versus-host disease (GvHD) and Crohn's disease: Pre-clinical experiments have shown that MSC infusions can be highly efficacious in GvHD but ineffective if administered before the onset of the disease or after permanent tissue damage has occurred, thus indicating that an acute inflammatory microenvironment is crucial to promote the therapeutic properties of MSCs [33]. The initial clinical experience with GvHD supports such a notion, because MSCs have no effect on the chronic form of the disease. Pre-clinical studies have extensively supported this notion [34-36] and more recently, a European Blood and Marrow Transplantation (EBMT) multicentre clinical trial has described the successful use of MSCs in severe steroid-resistant acute graft-versus-host disease (GvHD), with 60% of patients obtaining complete durable remissions without toxicity [37]. MSCs are currently being tested for the treatment of Crohn's disease based on the observation that transplanted bone marrow cells can repopulate the human gastrointestinal tract and that BM-derived epithelial cells are remarkably increased during the epithelial regeneration after GvHD and ulcer formation [38].

Clinical experience of MSC treatment in MS: There are reports of small numbers of patients with MS who received IV or intrathecal (IT) infusions of MSC cells with some reported benefit. The results of an early phase I/II study of MSCs in neurological diseases reported on the administration of autologous MSCs to 19 amyotrophic lateral sclerosis and 15 MS patients [39]. MSCs were given as a combination of IT and IV injections, at doses up to 60–70x10^6 cells per injection per patient and patients were followed for 6–28 months with the main purpose being to determine feasibility and safety. No patient experienced significant side effects except that of mild meningeal irritation, having symptoms of headache and fever, in those receiving IT injections of cells. Magnetic

resonance imaging (MRI) in 20 of the patients did not reveal any unexpected pathology 1 year after MSC transplantation. No injection-related (early or late) infections were reported. Additional data on the use of MSCs in MS included a small study in 10 patients with MS from Iran, which reported no significant adverse events [40]. A MRC funded phase IIa study (with MS Society addendum funding) by the applicants (SC & DHM) had also treated 10 patients. The importance of this latter study with regard to the current proposal is twofold: (1) it had established capability for cell expansion, validation, regulatory screening of autologous MSCs with successful intravenous delivery of autologous MSCs to 10 patients without any adverse events; and (2) the patients had progressive disease unlike the proposed study that is targeted at an "inflammatory-dominant" cohort with clinically and radiologically highly active disease. Thus we would regard the two studies as being complementary - with the MRC study providing significant added value, leverage and field-tested expertise and methodologies e.g. standard operating procedures (SOPs) to ensure that the desired outcomes are realistic. We are satisfied that sufficient information on the safety of autologous bone marrow-derived, intravenously administered MSCs exists to propose exploring such therapy in patients with active inflammatory MS. This position appears justified by the very latest publication which was a randomised, double-blind, placebo-controlled trial of autologous mesenchymal stem cells in multiple sclerosis [41]. A similar crossover trial design was employed and the results in 9 patients, whilst not powered to detect significance, showed a trend to lower mean cumulative number of GELs at 6 months in those patients treated with MSCs. No serious adverse events were identified.

5.2.4. Rationale for this study

Evidence from preclinical studies suggested that MSCs were an effective cell therapy for experimental autoimmune encephalomyelitis (EAE). In EAE, the IV injection of MSCs ameliorates the clinical course of acute disease and decreases demyelination, immune infiltrates and axonal loss, but has little or no effect in later stages of EAE. These results led to the conclusion that MSC administration should focus on individuals with on-going inflammation and before they develop irreversible disability. Pioneer small clinical studies in MS have already suggested their safety, thus paving the road from their clinical exploitation into larger phase II studies addressing their biological effect on markers of disease activity [39]. An international panel of MS neurology and stem cell experts, as well as immunologists formed an "International Mesenchymal Stem Cells Transplantation" (MSCT) Study Group with the aim to derive a consensus on the utilisation of MSCs for the treatment of MS along with protocols for the culture of the cells and the treatment of patients. This international consensus, based on the efforts of Dr. Uccelli (University of Genoa) and Dr. Freedman (University of Ottawa), has been achieved and was published in 2010 [42]. In the consensus paper, the authors devised the synopsis of a phase I/II clinical trial, which will be fully adopted by scientists and clinicians belonging to the MSCT Study Group and presented to the respective national regulatory and funding agencies as single nation studies. This approach will enable the running of a series of parallel, independent but related, national studies adopting identical protocols, inclusion and exclusion criteria, primary and secondary outcomes collected on identical forms. The opportunity to pool the results of these studies will overcome the limitations of small phase I/II studies and provide enough statistical power to draw conclusions on the safety and efficacy of autologous MSCs transplantation in MS [42]. Our study will be conducted following the guidelines of the aforementioned consensus and utilise a protocol identical to the one adopted in other European countries and North America participating in this international collaborative effort. Identical, parallel but independent national trials will be conducted in Spain, Sweden, Denmark, France, Germany and Canada in order to assess the efficacy, safety and the toxicity of MSC infusion, as administered within this specific treatment protocol, in active MS. Evaluation of primary and secondary outcomes as well as exploratory outcomes will be carried out at the single national trial level but, more importantly, also on the entire data set obtained by pooling the results of all national trials, for an expected total of 160 randomised patients.

6. STUDY OBJECTIVES

6.1 PRIMARY STUDY OBJECTIVES

There are two co-primary objectives:

- To assess the safety of IV therapy with autologous MSCs in MS patients.
- To evaluate the activity of autologous MSCs in MS patients, quantified by the reduction in the number of new contrast-enhancing lesions (GEL) on MRI scans over a 24 week period.

6.2 SECONDARY STUDY OBJECTIVES

To gather preliminary information about the efficacy of the experimental treatment in terms of:

- combined MRI activity including MRI measures of remyelination
- clinical activity including incidence of relapses and disability progression and
- effect on immunological markers (for patients in the first phase of recruitment).

7. STUDY DESIGN

STREAMS is a randomised, double-blind crossover study comparing IV treatment with autologous MSCs vs. suspension media in MS patients with regards to the number of adverse events and new MRI activity (measured as GEL) at 24 weeks. A secondary analysis will compare the number of adverse events and GEL at weeks 0-23 and 24-48 within each treatment group (see Figure 1).

7.1 STUDY OUTCOME MEASURES

Primary endpoints:

- number, timing and severity of adverse events in the MSC treatment group will be compared to the placebo group;
- total number of GEL counted over month 1, 3 and 6 will be compared between treatment groups.

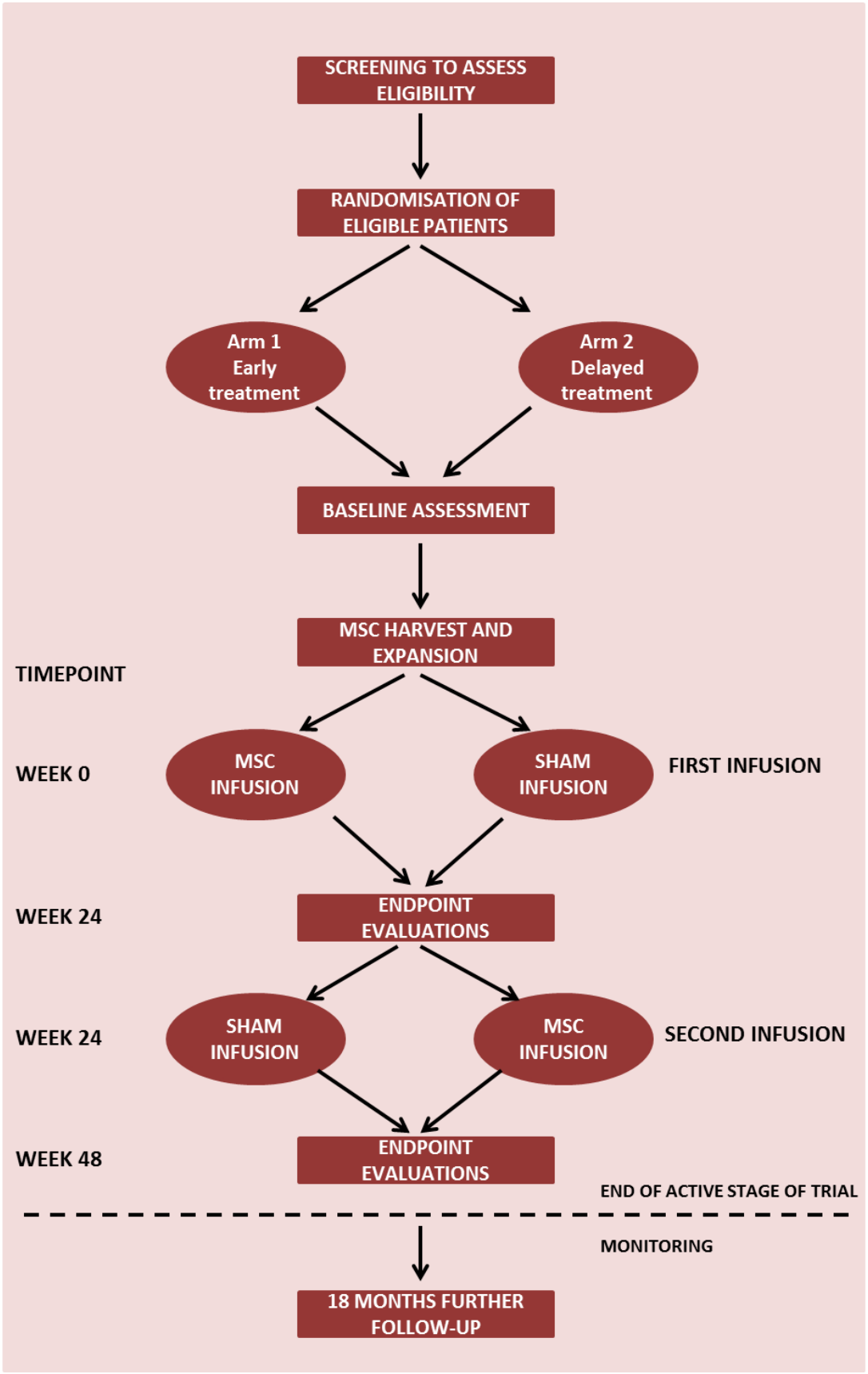
Secondary endpoints:

- volume of GEL identified over months 1, 3 and 6 will be compared between treatment groups.
- number of GEL counted over months 7, 9 and 12 (crossover re-treatment) compared between treatment periods (placebo vs. active treatment) for each patient.
- combined unique MRI activity (number of new or enlarging T2, or enhancing or re-enhancing lesions).
- number of relapses in MSC treatment group vs. placebo group in the first 6 months and after crossover re-treatment in the two groups (see below for the definition of a relapse).
- time to sustained progression of disability (RRMS group) (see below for definition of sustained EDSS progression) and proportion of progression-free patients.

- proportion of disease-free patients (i.e. patients without relapses) and progression of MRI activity in the two groups.
- changes in the Multiple Sclerosis Functional Composite (MSFC) score in MSC treatment group compared to the placebo group.
- effect of MSCs on the peripheral immune responses (for patients in the first phase of recruitment).
- effect of MSCs on delayed type hypersensitivity (Type 1V hypersensitivity) reaction as measured by the Mantoux test.

Given the exploratory nature of MSC use, no specific quantitative assumptions have been made about the outcomes measures.

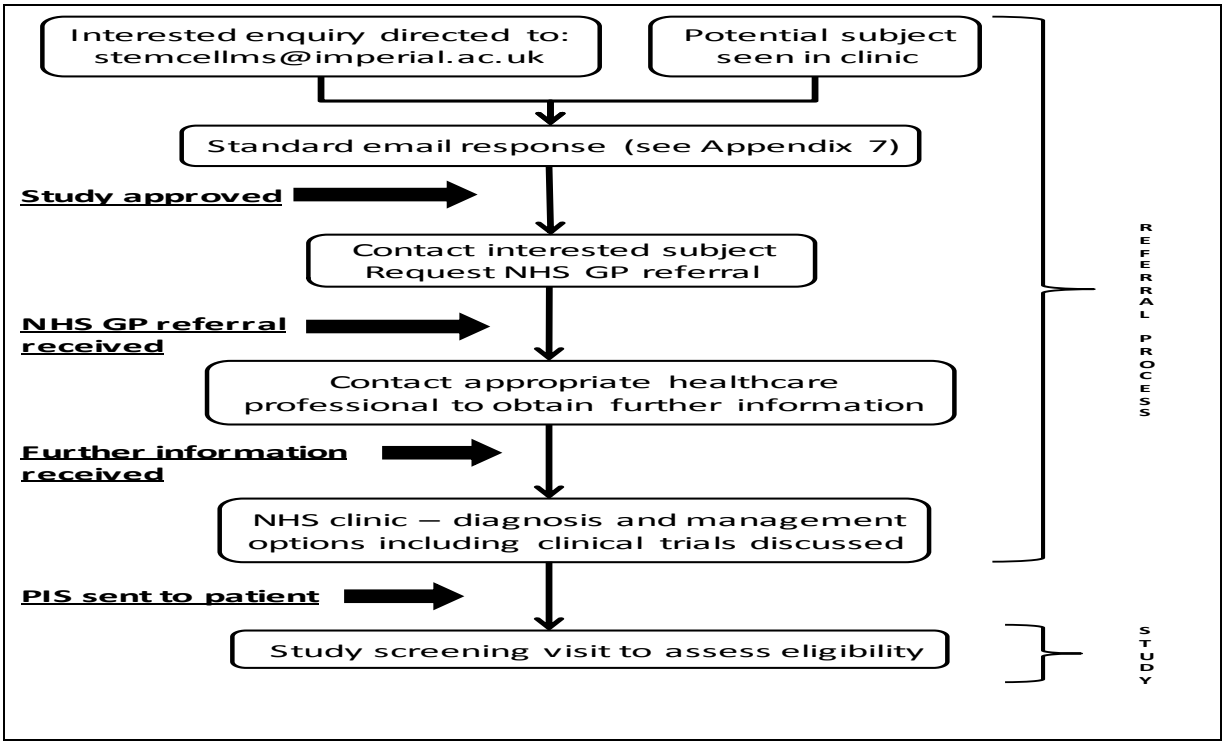
Figure 1: Patient flow and study outline



7.2 SUBJECT RECRUITMENT

- Patients recruited will have been seen by the Imperial College Healthcare NHS Trust MS service (Figure 2).
- Interested parties who enquired about the trial have been directed to an email address to register their interest: stemcellms@imperial.ac.uk. Patients have been sent a response described in the 'Stem Cell referrals' standard operating procedure (Appendix 7) to prevent subjects delaying appropriate therapies recommended by their current medical professional.
- When ethical approval is given, subjects will be contacted to determine:
  - Whether they are they still interested in taking part
  - Whether they fit the inclusion criteria that can be assessed over the phone
  - Whether they would be able to be referred to Imperial College Healthcare NHS Trust MS service for further evaluation if needed.
- On receipt of a NHS referral letter, the patient will be seen in the NHS clinic for an assessment of their MS. Prior to clinical review by Imperial College Healthcare NHS Trust MS service, subjects will be contacted to provide further details of their MS diagnosis, if not already provided, to consider the likelihood of there being further management options - including all ongoing trials – available to them as a consequence of that review.
- The review by the Imperial College Healthcare NHS Trust MS service will ensure that appropriate investigations have been performed to confirm the diagnosis of MS. Management options – including all available clinical trials – will be discussed with the patient. If appropriate, it will be confirmed that their MS care will be under Imperial College Healthcare NHS Trust otherwise their care will revert solely to their usual medical practitioner.

Figure 2: Patient eligibility selection and flow



Eligibility for the STREAMS study will only be considered at the study screening visit. The inclusion criteria will need to be fulfilled (see section 8.2).
At this visit, they will have a full discussion of the Patient Information Sheet (PIS) and if they consent, will proceed to undergo all study assessments and procedures including bone marrow harvesting (see Figure 4).

8. PARTICIPANT ENTRY

8.1 REFERRAL CRITERIA

All potential study patients need to be referred by a clinician and the referral should include:

1. A current diagnosis of MS.
2. Age 18-50.
3. An assessment of mobility level (preferably EDSS score) documenting EDSS 2.0-6.5 or equivalent mobility.
4. MRI brain and/or spinal cord performed within the last 18 months with findings consistent with MS and showing at least one gadolinium-enhancing lesion (GEL) or one new lesion compared to previous imaging.

Justification for the referral criteria

Criterion 1
Given the experimental nature of the intervention, patients with only one symptomatic episode (diagnosed MS on grounds of paraclinical support) will not be considered.
All patients with clinically defined MS will need to satisfy the revised McDonald criteria.

Criterion 2
The lower limit of age has been set because MS in children is rare and differs significantly from MS in the adult population, and because the study will be carried out in adult (not paediatric) hospital facilities. The upper limit of age has been specified for safety of the experimental cell therapy intervention and to limit the confounding effect of age-related differences in spontaneous lesion repair in the study population.

Criterion 3
A lower Expanded Kurtzke Disability Status Score (EDSS) limit has been specified to ensure that recruited patients have acquired sufficient disability to merit experimental therapeutic approaches.
An upper Expanded Kurtzke Disability Status Score (EDSS) limit has been specified to ensure that recruited patients are able to tolerate the trial procedures and assessments.

Criterion 4
This provides a group which will reduce the chance of unnecessary patient testing to meet MRI requirements for the inclusion criteria.

8.2 INCLUSION CRITERIA

Patients with clinically and radiologically active multiple sclerosis as defined by:

1. Diagnosis of MS:
   a. Relapsing remitting MS (RRMS): ≥1 moderate-severe relapse **and** ≥1 GEL in past 18 months **or** ≥1 moderate-severe relapse and ≥1 new T2 lesion in past 18 months.
   b. Secondary progressive MS (SPMS) with an increase of ≥1 EDSS point (if baseline EDSS ≤ 5.0) or 0.5 EDSS point (if baseline EDSS ≥5.5), in the previous 18 months **and** ≥1 GEL in past 18 months **or** ≥1 moderate-severe relapse and ≥1 new T2 lesion in past 18 months.
   c. Primary progressive MS (PPMS) patients with positive oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) and an increase of ≥1 EDSS point (if base-line EDSS ≤ 5.0) or 0.5 EDSS point (if baseline EDSS ≥5.5), or quantifiable, objective evidence of equivalent progression in the previous 18 months **and** ≥1 GEL in past 18 months or ≥1 new T2 lesion in past 18 months.
2. Age 18 to 50 years.
3. Disease duration 0 to 10 years from diagnosis (inclusive).
4. EDSS 2.0 to 6.5 at screening evaluation.
5. ≥1 GEL on MRI within 6 months prior to harvesting.
6. Adequate culture of a subject's MSCs and their release for clinical use.

Justification for the inclusion criteria

Working definitions for active MS emphasise accumulation of disability, occurrence of relapses or a combination of both. Our aim is to identify a patient population who is at increased risk of continued progression and loss of function but has not accumulated advanced levels of neuronal injury or degeneration which could fail to show a treatment effect even if it exists. We have therefore formulated criteria to recruit patients with mild to moderate disability and evidence of ongoing inflammation (gadolinium-enhancing lesions) relatively early in the disease.

Each subject must generate MSCs suitable for clinical use otherwise the procedure will have no chance of benefit (section 10.3).

8.3 EXCLUSION CRITERIA

1. RRMS without at least one severe relapse in the previous 18 months or without at least one GEL or one new T2 in the previous 18 months.
2. SPMS without relapses and without new lesions (GEL or T2 positive) on MRI in the last 18 months.
3. PPMS without positive CSF OCBs or without a GEL or new T2 lesion in the previous 18 months.
4. No gadolinium enhancing lesion(s) in the 6 months prior to bone marrow harvesting.
5. A previously ineligible patient who failed to meet the MRI requirements of the inclusion criteria will not be reviewed unless further imaging, revealing ≥1 GEL, becomes available.
6. Failure of BM sample to generate MSCs suitable for clinical use within a specified time frame.
7. Treatment with any immunosuppressive therapy, including natalizumab and fingolimod, within the last 3 months.
8. Treatment with interferon-beta or glatiramer acetate within the last 1 month.
9. Treatment with alemtuzumab (campath-1H) within the last 2 years.

10. Prior treatment with total lymphoid irradiation and autologous or allogeneic hematopoietic stem cell transplantation.
11. Participation in clinical trials of any experimental drugs in the 6 months before study entry.
12. Corticosteroid treatment in the previous 30 days.
13. Presence of any active or chronic infection.
14. Previous history of a malignancy other than basal cell carcinoma of the skin and carcinoma in situ that has been in remission for more than one year.
15. Severely limited life expectancy by any other co-morbid illness.
16. Abnormal blood counts, a history of myelodysplasia or other cytopenia.
17. Known pregnancy, positive urine pregnancy test at screening or risk or pregnancy (this includes patients who are unwilling to practice active contraception for the duration of the study).
18. Any breastfeeding woman.
19. Contraindication to MRI including but not limited to intracranial aneurysm clips (except Sugita), history of intra-orbital metal fragments that have not been removed by an MD (as confirmed by orbital X-Ray), pacemaker and non-MR compatible devices (e.g. heart valves, inner ear implants), history of claustrophobia or the inability of the subject to lie still on their back for a period of 1.5 hours in the MRI scanner.
20. An estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2 or history or presence of renal impairment (e.g. serum creatinine clearance less than 30ml/min).
21. Inability to give written informed consent/comply with study procedures.
22. Any significant organ dysfunction or co-morbidity that the Investigators consider would put the subject at unacceptable risk by participating in the study or that would interfere with the functional assessments.

Justification for the exclusion criteria

These criteria are used to exclude MS patients without an active, inflammatory disease and to exclude patients with medical problems or those who are receiving or have received treatments that could increase their risk of serious morbidity or mortality from MSCT or any other facet of this protocol.

8.4 CONCOMITANT MEDICATIONS

All concomitant medications will be recorded at every study visit. Treatment with disease-modifying therapies is not permitted throughout the study – and appropriate wash-out periods are required (see section 8.3) prior to enrolment. Use of corticosteroids will be permitted for relapses that occur during the study although patients cannot have received corticosteroids in the 30 days prior to enrolment. In addition, in the event of a relapse occurring either between randomisation and the first infusion (Week 0) or before the second infusion (Week 24), the infusion will be delayed if the relapse requires the use of corticosteroids, so ensuring that there will be at least 30 days between the methylprednisolone and the infusion. Relapses and the corticosteroids received will be documented on the electronic case report forms.

Symptomatic treatments for MS can be continued throughout the study and may also be initiated if appropriate and at the usual doses. If a patient is already being treated with Fampridine (Fampyra®) at the screening visit, this can be continued but this treatment cannot be initiated during the course of the trial because the effects on walking will affect some clinical endpoints being measured.

Symptomatic treatments for MS permitted in the STREAMS trial (this list may not be exhaustive):

Common Symptoms in MS	Examples of drugs used to treat symptom
Fatigue	Modafinil, amantadine
Depression	Tricyclic antidepressants, monamine-oxidase inhibitors, selective serotonin reuptake inhibitors, other antidepressant drugs.
Spasticity	Baclofen, tizanidine, dantrolene, diazepam, gabapentin, botulinum toxin
Reduced walking ability	Fampridine (Fampyra®)*
Bladder – urinary urgency, frequency and incontinence	Anti-cholinergics (e.g oxybutynin, tolterodine, darifenacin, solifenacin)
Constipation	All types of laxatives
Oscillopsia	Gabapentin, baclofen, clonazepam
Erectile dysfunction	Phosphodiesterase type 5 inhibitors (e.g. sildenafil)

*Fampridine can be continued if it was prescribed before enrolment into the trial but cannot be initiated during the study.

8.5 WITHDRAWAL CRITERIA

Subjects can voluntarily withdraw at any time from the study and follow-up assessments. All data collected up to that point will be retained. Any collected biological specimens will be kept and used for research unless subjects request that these unused samples are destroyed.
A patient's participation in the study may also be discontinued at any time at the discretion of the investigator, and justifiable reasons include Suspected Unexpected Serious Adverse Reactions (SUSAR) or deterioration in the disease activity requiring immunomodulatory treatment but their data until that point will be included in the analysis.

9. RANDOMISATION AND ENROLMENT PROCEDURE

9.1 ELIGIBILITY FOR RANDOMISATION

To be eligible for randomisation a subject must have fulfilled all the entry criteria.

9.2 RANDOMISATION PRACTICALITIES

All subjects will be randomised after the investigator has verified that they are eligible by the criteria (section 8). Randomisation will be carried out within a web-based system, made available to authorised researchers, by the Clinical Research Organisation (CRO) in charge: *Centro Clinical Trials, Fax +39/010354103 Email:* clinicaltrials@hsanmartino.it

Eligible patients will be randomised to receive immediate vs. delayed treatment with either autologous MSCs or equivalent volume of suspension media at baseline. At 6 months, patients and investigators will continue to be blinded to therapy, but treatments will be reversed (i.e. those who received initial MSCs will receive suspension media and vice versa).

9.3 BLINDING PROCEDURES

The patient, assessor team (EDSS, MSFC, MSIS-29, SF36) and MRI team will be blinded to the treatment assignments.
The treating team will not be blinded to treatment assignments. For details about roles, see the Delegation Log.

9.4 UNBLINDING

Unblinding is restricted to clinically relevant needs impacting patient safety. It should be used only under circumstances where the knowledge of the treatment is necessary for the proper care of the patient. If the treatment code is broken, the reason and date should be recorded and signed by the investigator. If treatment is discontinued prematurely for any reason other than disease progression the study data centre (Centro Clinical Trials) should be informed immediately. All patients will continue to be followed-up, irrespective of whether they have discontinued treatment prematurely.

9.4.1. Reasons for unblinding

Clinical events*:* This includes the occurrence of fatal or life-threatening SAE, or any circumstances wherein the judgment of the treating neurologist, blind to the treatment modality, may compromise patient safety or where the patient is unable to provide informed consent to clinically necessary investigations, procedures or treatments as a consequence of the blinding.
MRI findings: Any radiological evidence suggestive of anomalous disease exacerbation or adverse event. This refers to an increase of ≥5 number of lesions above the number of lesions on the baseline scan, or the appearance of atypical lesions as assessed by the reviewing radiologist (section 14.1.1). The treating team will be informed as soon as possible.

10. STUDY TREATMENTS

10.1 BONE MARROW HARVEST

Pre sampling checks:
Coagulation studies will be performed at the Week -10 and Week 0 visit (screening and pre-treatment, respectively). In accordance with JACIE (**J**oint **A**ccreditation **C**ommittee ***I****nternational Society for Cellular Therapy – the European Group for Blood & Marrow Transplantation*) requirements, participants will be tested at a maximum of 30 days before bone marrow and skin sampling for HIV 1, HIV 2, Hepatitis B, Hepatitis C, HTLV 1, HTLV 2, CMV, and Syphilis. These latter tests will be done at the Week -10 visit.

Bone marrow sampling:
Bone marrow (BM) will be harvested at week -8 according to the Trust policy. The principle is to collect only the BM rich in stroma and early precursors which are contained in the first part of the aspirate. Therefore, the procedure consists of repetitive extractions of 2 ml of BM aliquots. The procedure will require local anaesthesia and intravenous sedation may be required in selected cases. The procedure can be done as an outpatient in the haematology day-care unit. The entire methodology and SOPs have been established and validated in ten MS patients in Cambridge and in 15 patients with GvHD at Hammersmith Hospital.
The bone marrow aspiration will be performed by the registrar in charge of aspirates according to the SOP for mesenchymal stem cell preparation.

10.2 ISOLATION AND EXPANSION OF AUTOLOGOUS MSCS

The Haematology Laboratory (based at Imperial College London), will receive the bone marrow and will act as the manufacturing site for the trial. It will produce the MSCs under the existing GMP license and according to the manufacturing protocol as described in the IMP dossier submitted to the MHRA.

Briefly, MSCs are isolated from bone marrow harvests and *ex-vivo* expanded in the presence of Modified Eagles Medium supplemented with 5% human platelet lysate according to a Standard Operating Procedure included in the MHRA IMP dossier. The final product is tested to exclude growth of aerobic and anaerobic bacteria and defined as adherent cells positive for CD73, CD90 and CD105 and negative for CD45.

Adequate expansion in culture to obtain sufficient number of MSC to achieve the treatment dose is a prerequisite for continuation in the trial. MSC preparations that have not expanded to confluency (to reach a target of 1-2 million cells/kg body weight) within 52 days from the start of culture will be aborted and the subject will be withdrawn from the study. If the expansion is <1 million cells/kg body weight, then these cells will be stored in the biobank at Imperial College London. Consent for these actions will have been sought at the screening visit.

10.3 RELEASE OF THE MSC PRODUCT FOR CLINICAL USE

MSCs may be released from the laboratory for clinical use if they fulfill the provisions of the advanced therapy medicinal products (ATMP) license described in the dossier approved by the MHRA.
MSC preparations that have not passed the release criteria will be disqualified for trial clinical use and the subject will be withdrawn from the study.

10.4 STUDY TREATMENT INFUSION OF AUTOLOGOUS MSCS OR PLACEBO

At week 0 and at week 24, a single infusion of either *ex-vivo* expanded autologous MSCs or suspension media will be administered intravenously. For MSCs, we will give a dose of 1-2 x 10^6 MSC/Kg recipient body weight (as measured at baseline).

10.5 POST-STUDY TREATMENT INFUSION EVALUATION

Following the study treatment, patients will be monitored for infusion-related toxicity for three hours. Temperature, blood pressure, pulse and oxygen saturation will be measured at baseline and after 15 minutes, 60 minutes and 3 hours. Any toxicity will be treated at the discretion of the attending physician.

10.6 TOXICITY ASSESSMENT

Infusion-related toxicity is defined as any of the following within three hours post-treatment infusion:

- Systolic blood pressure (mmHg) <90 or >180, or >33% decrease or increase from baseline.
- Diastolic blood pressure (mmHg) <50 or >100, or >33% decrease or increase from baseline.
- Heart rate: <60 or >110 or >33% decrease or increase from baseline.
- Body temperature (°C) >38.5 or >1°C increase.
- Any other toxic side effect.

11. PHARMACOVIGILANCE

11.1 DEFINITIONS

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical trial subject, administered a medicinal product and, which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.*

Adverse Reaction (AR): All untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey, in general, that there is evidence or argument to suggest a causal relationship.*

Unexpected Adverse Reaction: An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product*). When the outcome of the adverse reaction is not consistent with the applicable product information, this*

adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.

Serious Adverse Event (SAE) or **Serious Adverse Reaction:** Any untoward medical occurrence or effect that at any dose:

- Results in death

- Is life-threatening – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- Requires hospitalisation, or prolongation of an existing inpatient hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

11.2 CAUSALITY

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below. If any doubt about the causality exists, the local investigator should inform the study coordination centre which will notify the Chief Investigator. Other clinicians may be asked to advise in some cases. In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship.
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

11.3 REPORTING PROCEDURES

All adverse events should be reported (Figure 3). Depending on the nature of the event, the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. A flowchart is given below to aid with the reporting procedures.

11.3.1. Non-serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the relevant AR/AE.

11.3.2. Serious AR/AEs

Fatal or life threatening SAEs and SUSARs should be reported on the day that the local site is aware of the event. The SAE form requests the following information on any event: the nature of the SAE, the date of onset, the severity, corrective therapies given, the outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

SAEs

An SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours. However, death from an unrelated cause (e.g. road traffic accident), MS relapses and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

- Complete the SAE case report form & send it as soon as possible (within 24 hours and preferably by fax), signed and dated to the study coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

- Contact the study coordination centre by phone and then send the completed SAE form to the study coordination centre within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Figure 3. Safety reporting overview

Contact details for reporting SAEs and SUSARs
Fax: xxx, attention xxx
Please send SAE forms to: xxx
Tel: xxx (Mon to Fri 09.00 – 17.00)

Figure 4. Schedule of study assessments and treatments

	Screening - 10 weeks (±7 days)	- 8 weeks (±7 days)	- 8 weeks (±7 days)	- 2 weeks (±14 days)	Week 0	Week 0	Week 4 (±7 days)	Week 8 (±7 days)	Week 12 (±7 days)	Week 16 (±7 days)	Week 20 (±7 days)	Week 24 (±7 days)	Week 24 (±7 days)	Week 28 (±7 days)	Week 32 (±7 days)	Week 36 (±7 days)	Week 40 (±7 days)	Week 44 (±7 days)	Week 48 (±7 days)	Week 48 (±7 days)
Informed Consent	x	x																		
Inclusion/exclusion review	x	x			x															
Medical history	x																			
Concomitant medications record	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical exam	x	x			x		x		x		x	x		x		x		x	x	
Study treatment (MSC) infusion					**x**							**x**								
Adverse events record		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Release of MSC product				x																
Randomisation																				
Lumbar puncture		x									x							x		
Skin biopsy*		x																		
Bone marrow harvest		x																		
EDSS	x	x			x	x	x		x			x	x	x		x			x	
MSIS29	x				x		x		x			x		x		x			x	
MSFC	x				x							x							x	
T25-FW	x				x		x		x			x		x		x			x	
Quality of life/Fatigue impact scale	x				x		x		x			x		x		x			x	
Hematology, Blood Chemistry	x				x		x		x		x	x		x		x		x	x	
Blood tests pre-bone marrow harvest	x																			
Pregnancy test	x				x		x		x		x	x		x		x		x	x	
Urinalysis	x				x		x		x		x	x		x		x		x	x	
Research blood samples*		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Mantoux skin test		x			x							x							x	
Mantoux readout			x			x							x							x
MRI					x		x		x			x		x		x			x	
ECG	x																			

* Only part of the first phase of recruitment

12. ASSESSMENT AND FOLLOW-UP

12.1 STUDY ASSESSMENTS

The trial will run for 48 weeks from the patient's baseline visit. There will be a screening visit 10 weeks before the scheduled baseline visit where informed consents will be taken and the patient's eligibility assessed. Patients will be informed that they may withdraw consent at any point throughout the trial should they wish to do so and that their subsequent treatment will not be compromised by their withdrawal.

12.2 SCHEDULE OF VISITS

At scheduled visits, vital signs and adverse events will be recorded for every patient. Other procedures will be performed according to the study schedule as depicted in Figure 4.

12.2.1 Study visits and treatment schedule

Minus 10 weeks ± 7 days
Subjects will be assessed for eligibility and will have the following performed:

- Sign informed consent to enter the study.
- Sign MRI consent form.
- Full medical and MS history will be taken.
- Concomitant medications will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- A pre-bone marrow sampling blood screen will be performed in accordance with JACIE (**J**oint **A**ccreditation **C**ommittee ***I**nternational Society for Cellular Therapy – the **E**uropean Group for Blood & Marrow Transplantation*) requirements - participants will be tested for HIV 1,HIV 2, Hepatitis B, Hepatitis C, HTLV 1, HTLV 2, CMV, and Syphilis. Blood tests for HIV, Hepatitis B and C will be mentioned specifically on the consent form and we will ensure that subjects are adequately counselled so that they fully understand the implications of a positive test.
- Urinalysis will be performed.
- EDSS, MSFC, MSIS-29 and SF36 assessments will be carried out.
- A baseline 12 lead ECG (section 8.7.5).
- Randomisation to decide order in which the MSCs and dummy infusion will be given.

Minus 8 weeks ± 7 days
Subjects will be admitted as a day case to Hammersmith Hospital. The subjects will have the following performed:

- Informed consent will be confirmed.
- Inclusion/exclusion criteria will be reviewed.
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- An EDSS assessment will be carried out.

- 80 ml blood research samples will be taken (section 12.7.5).*

Subjects will then undergo four procedures:

- Bone marrow harvesting under sedation (section 12.7.1)
- Skin biopsy (section 12.7.2)*
- Lumbar puncture (section 12.7.3)
- Mantoux test (section 12.7.6)

If successfully completed, MSC manufacturing will commence and the subjects will proceed further in the study.

Minus 8 weeks ± 7 days +48/72 hours

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- Reading of the Mantoux test.
- 80 ml blood research samples will be taken.*

Minus 2 weeks ± 14 days

This is a virtual visit not requiring patient attendance and defines a time point for MSC release. If the manufactured MSC product is released for clinical use, subjects will be eligible to continue in the study.

Week 0

Subjects will have the following performed:

- An MRI will be carried out **prior to** infusion of the study treatment (section 12.8).

Subjects will then be admitted as a day case to Hammersmith Hospital.

- Inclusion/exclusion criteria will be reviewed especially to confirm that inclusion criteria 6 is fulfilled.
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- A Mantoux test will be performed.
- Urinalysis will be performed.
- EDSS, MSFC, MSIS-29 and SF36 assessments will be carried out.
- The study treatment will be infused.

Week 0 +48/72 hours

Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.

- An EDSS assessment will be carried out.
- 80mls of blood research samples will be taken.*
- Reading of the Mantoux test.

Week 4 ± 7 days
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- Urinalysis will be performed.
- EDSS, T25-FW, MSIS-29 and SF36 assessments will be carried out.
- An MRI will be carried out.

Week 8 ± 7 days
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- 80 ml blood research samples will be taken.*

Week 12 ± 7 days
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- Urinalysis will be performed.
- EDSS, T25-FW, MSIS-29 and SF36 assessments will be carried out.
- An MRI will be carried out.

Week 16 ± 7 days
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- 80 ml blood research samples will be taken.*

Week 20 ± 7 days
Patients will be admitted as a daycase and have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- Urinalysis will be performed.
- A lumbar puncture will be performed.
- 80 ml blood research samples will be taken.*

Week 24 ± 7 days
Subjects will have the following performed:

- An MRI will be carried out **prior to** infusion of the second study treatment.

Subjects will then be admitted as a day case to Hammersmith Hospital.

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- A Mantoux test will be performed.
- Urinalysis will be performed.
- EDSS, MSFC, MSIS-29 and SF36 assessments will be carried out.
- The study treatment will be infused.

Week 24 ± 7 days + 48/72 hours
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- An EDSS assessment will be carried out.
- 80mls of blood research samples will be taken.*
- Reading of the Mantoux test.

Week 28 ± 7 days
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- Urinalysis will be performed.
- EDSS, T25-FW, MSIS-29 and SF36 assessments will be carried out.
- A MRI will be carried out.

Week 32 ± 7 days
Patients will have the following performed:
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- 80 ml blood research samples will be taken.*

Week 36 ± 7 days
Patients will have the following performed:
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*
- Urinalysis will be performed.
- EDSS, T25-FW, MSIS-29 and SF36 assessments will be carried out.
- A MRI will be carried out.

Week 40 ± 7 days
Patients will have the following performed:
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- 80 ml blood research samples will be taken.*

Week 44 ± 7 days
Patient will have the following performed:
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- Urinalysis will be performed.
- A lumbar puncture will be performed.
- 80 ml blood research samples will be taken.*

Week 48 ± 7 days
Patients will have the following performed:
- A MRI will be carried out.

Subjects will then be admitted as a day case to Hammersmith Hospital.
- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will undergo a full physical examination including vital signs.
- Blood will be taken for haematological and clinical chemistry parameter measurement.
- Pregnancy testing will be performed (if applicable).
- 80 ml blood research samples will be taken.*

- A Mantoux test will be performed.
- Urinalysis will be performed.
- EDSS, MSFC, MSIS-29 and SF36 assessments will be carried out.

Week 48 ± 7 days + 48/72 hours
Patients will have the following performed:

- Concomitant medications will be recorded.
- Adverse events will be recorded.
- The patient will have their vital signs checked.
- 80mls of blood research samples will be taken.*
- Reading of the Mantoux test.

*** Only for the patients in the first phase of recruitment**

Conclusion of the active stage of the trial
Once a patient has finished the active stage of the study, they will continue to be followed-up on the NHS for at least 2 years after the last study drug administration. They will be offered appropriate treatment according to best medical practice.

12.2.2. Unscheduled visits

If subjects have a significant relapse (defined as one which affects the EDSS), an unscheduled visit will need to take place as soon as possible. Minor relapses should be reported at the next scheduled visit. In addition, if adverse effects develop which require clinical evaluation, unscheduled visits will be arranged and patients will be assessed by a treating clinician and managed as medically appropriate.

12.3 EVALUATION OF MS

Relapses will be assessed according to the protocol definition. Disability will be assessed using the Expanded Disability Status Scale (EDSS) rating scale (Appendix 1).

12.3.1. MS relapses

Only relapses meeting the protocol definition will initially be determined, but a separate analysis will also include relapses not meeting the protocol definition.
Definition of a relapse: any perceived change in symptoms or signs that persist for ≥ 48 hours in the absence of flu or fever and is accompanied by objective changes on EDSS functional subscores (FSS) that leads to either a 1.0 point change in EDSS (for baseline EDSS 2.0 - 5.0) or a 0.5 point change (for baseline EDSS ≥ 5.5) together with FSS changes of 2 points in any one FSS or 1 point in any 2 FS.

12.3.2. Progression of disability

Definition of EDSS progression is any 6-month sustained increase in EDSS:

- For baseline EDSS <5.5, any 1 point increase in EDSS.
- For baseline EDSS ≥ 5.5, any 0.5 point increase in EDSS.

12.4 LOSS TO FOLLOW-UP

Subjects will be under the governance of the clinicians running the trial. The GP will be contacted and permission sought to contact subjects by phone.

12.5 TRIAL CLOSURE

12.5.1. Premature closure of the study

This study may be prematurely terminated, if in the opinion of the investigator, there is sufficient reasonable cause. The terminating party will provide written notification documenting the reason for study termination to the investigator. Circumstances that may warrant termination include, but are not limited to:

- Treatment related mortality exceeding 30% at 24 hours from the beginning of the treatment.
- Determination of unexpected, significant, or unacceptable risk to patients.
- Failure to enter patients at an acceptable rate.
- Insufficient adherence to protocol requirements.
- Insufficient complete and/or valuable data.
- Protocol violation.
- Administrative decision.
- New data able to influence the willingness of the investigators to pursue the study

12.5.2. Closure of the study after completion

Once a subject has completed 48 weeks of the study they will be considered to have completed the trial. The study will be closed when all participants have completed all study assessments and all regulatory requirements have been fulfilled.

12.6 CLINIC-BASED ASSESSMENTS

12.6.1. EDSS

The EDSS will be assessed regularly throughout the study. It will be performed by a neurostatus trained doctor (Appendix 1).

12.6.2. MSIS-29

The MSIS-29 is a self-assessment questionnaire that is used to assess disability in patients with MS. Patients may use a computer-based version of the MSIS-29 which they will complete online at the scheduled visits. In the event that the computer version is not available at the time of the clinic visit, the patient will be asked to complete a paper version at the clinic (Appendix 2).

12.6.3. MSFC

The MSFC is a combination of a timed walk, a nine-hole peg test and the Paced Auditory Serial Addition Test (PASAT) and will be undertaken by the EDSS assessor or a trained nurse. Two practice sessions will be completed at the screening visit to account for learning effects (Appendix 3).

12.6.4. SF-36

The Short Form 36 (SF-36) has been designed to measure the quality of life of a patient. Some of the questions deal with physical parameters such as walking and climbing stairs etc whilst others deal with aspects such as extent of pain, mental aspects such as mood and an assessment of how a patient's condition affects their social life (Appendix 4).

12.7 OTHER STUDY EVALUATIONS

12.7.1. Bone marrow harvest

Bone marrow aspiration will be performed by the trained Registrar in charge of aspirates according to the SOP for mesenchymal stem cell preparation. Bone marrow will be collected under local anaesthesia and sedation will be administered if required.

12.7.2. Dermal tissue sampling*

A punch skin biopsy will be taken concurrently to the bone marrow procedure during a single Daycare attendance at the Hammersmith Hospital. The biopsy sample will be placed in cell culture medium and a same-day courier will ship it to the research laboratory in an approved container.

The skin biopsy will provide the opportunity to generate induced pluripotent stem cells (iPSCs) through manipulation of adult somatic cells for *in-vitro* research studies aiming to model the *in-vivo* treatment effect of MSCs. Generation of patient specific lines with neuronal derivatives using such factors has been recently reported for a range of neurological disorders, including derivation of motor neurons from patients with inherited MND and spinal muscular atrophy. Here we will generate neurons from a sample of recruited patients in order to establish an *in-vitro* assay of neuronal injury and putative protection.

IPSCs will be generated from patient dermal fibroblasts obtained from skin biopsy. We will generate highly enriched central neurons in order to use an in vitro disease model assay to test (i) the effects on IPSC-derived cultured neurons of autologous PBMCs (obtained pre-MSC infusion) alone and with co-culture of potentially protective autologous MSCs; and (ii) the effects on IPSC-derived cultured neurons of autologous PBMCs (obtained post-MSC infusion). As a further mechanistic refinement we will also test (iii) the effects on IPSC-derived cultured autologous neurons of pre-MSC infusion-derived antigen-specific, activated Th1, Th17 and cytotoxic cell lines alone and with co-culture of autologous MSCs. The prediction is that pre-MSC stimulated PBMCs and pro-inflammatory T helper cells will be injurious on IPSC-derived neurons and that this will be ameliorated by co-culture with MSCs, owing to their anti-inflammatory, neuroprotective actions.

* Only for the patients in the first phase of recruitment

12.7.3. Lumbar puncture (LP)

A diagnostic lumbar puncture for CSF analysis will be performed, by a trained clinician, during the Daycase visit in accordance with the NHS Trust Guidelines for LP.

Standard investigations will be performed including cell count, protein, glucose and oligoclonal bands to assess the clinical effects of MSCs on the CSF inflammatory activity. We will also aim to collect samples for further analysis including T- and B-cell receptor repertoire studies and for biomarkers including markers of neuronal damage.

12.7.4. Electrocardiogram (ECG)
A standard 12-lead ECG will be obtained at the screening visit.

12.7.5. Research blood samples*
Blood samples will be taken at the indicated time points to carry out immunological mechanistic studies aimed at assessing the effects of MSC infusion on peripheral blood immune cell subsets as well as the effects on immune responses potentially associated with disease pathogenesis or progression.
To characterise changes following MSC infusion in phenotypically defined T, B, NK, monocyte and dendritic cell populations *ex-vivo*, we will utilise a flow cytometric panel that also allows the phenotypic identification and enumeration of putative pro-inflammatory cell subsets. Immunological studies will include comparison of the pre- versus post-MSC infusion *in-vitro* T cell responses (proliferation, cyto- and chemokines) against a group of relevant antigens including viral and myelin proteins. All immunological assays have been employed successfully in other studies.

* Only for the patients in the first phase of recruitment

12.7.6. Mantoux tuberculin skin test (TST)
This will be performed to provide an *in-vivo* method of assessing the effect of the mesenchymal stem cells. It will be carried out at 4 time-points: pre-infusion (-8 weeks) and then with each subsequent infusion (Weeks 0 and 24) and at the end of the trial (Week 48). The Mantoux test, whilst typically a standard test of determining whether a person is infected by Mycobacterium tuberculosis, is being used to allow measurement of the delayed type hypersensitivity or cell-mediated immunity which causes tissue injury.
It involves introducing an antigen intradermally over the forearm – typically 2 TU (tuberculin units) of Statens Serum Institute (SSI) tuberculin RT23 in 0.1mls of solution - which generates a wheal 6-10mm in diameter. The induration, measured in millimetres, is read 48-72 hours later.

12.8 MRI PROCEDURES

12.8.1. Outline
All patients will be scanned at one site: the MS Society-funded 3T MRI scanner at the UCL Institute of Neurology. Scans will be acquired at weeks 0, 4, 12, 24, 28, 36 and 48. If drop-out occurs before the study ends, all remaining MRI scans will be obtained, if possible, at the scheduled time-points or if this will not be possible, at an unscheduled visit (exit visit). The MRIs will be analysed by Professor Miller's group at the Institute of Neurology but as the data will also contribute to the international collaborative effort, these MRIs will be anonymised and sent to the Medical Image Analysis Centre (MIAC), located in Basel, Switzerland which is being used for analysis of the primary outcomes.

12.8.2. Data management
Site qualification: dummy run procedure
As required by the Central MRI Analysis Facility in Basel.
Scan labelling
As required by the Central MRI Analysis Facility in Basel.
Data transfer
Images required for local analysis will use a DICOM to DICOM server transfer (peer to peer)

Quality control of images

Images required for local analysis will undergo a rigorous quality control (QC) process. This will ensure that scans have been performed using the study imaging protocol and to ensure that scans are suitable for analysis. For MTR this will include a segmentation and registration to check that the MTR scans have been acquired consistently and are biologically plausible.

12.8.3. MRI acquisition protocol

1. Conventional T2//T1/Gd sequences
   As required by the Central MRI Analysis Facility in Basel
2. 3D T1 sequences
   A volumetric T1-weighted scan of the brain at 1x1x1 mm^3 resolution will be acquired, covering the whole brain. The total acquisition time will be of the order of 5 minutes.
3. Magnetisation Transfer Imaging
   Images with MT-weighting on and off will be acquired, with full brain coverage and matching the voxel size of the 3D T1 protocol, at a resolution of 1x1x1 mm^3. This data will allow MTR maps to be calculated. Total scan time will be of the order of 20 minutes.
4. Diffusion-Weighted Imaging
   Diffusion-weighted data of the whole brain will be acquired for advanced diffusion analysis methods. Voxel size will be of the order of 2x2x2 mm^3 and cardiac gating will be employed to reduce physiological noise contamination. The total acquisition time will depend on the heart rate of the subject and will be of the order of 25 minutes.

12.8.4. Outcome measures

1. Cumulative number of new Gad-enhancing lesions weeks 4-24 inclusive (primary outcome measure to be analysed centrally by Dr Radu's group in Basel)
2. Cumulative number of new Gad-enhancing lesions weeks 28 to 48 inclusive
3. Cumulative number of persistent Gad-enhancing lesions weeks 4-24 and 28-48
4. Cumulative number of total Gad-enhancing lesions weeks 4-24 and 28-48
5. Volume of total Gad-enhancing lesions weeks 4-24 and 28-48
6. Cumulative number of new active lesions (new Gad-enhancing and new non-enhancing T2 lesions) weeks 4-24 and 28-48
7. Cumulative number of new and enlarging T2 lesions weeks 12 and 24 compared to baseline (week 0) and weeks 36 and 48 compared to week 24
8. Cumulative number of new T1 hypointense lesions on Gad-enhanced T1-weighted spin echo scans weeks 12 and 24 compared to baseline (week 0) and weeks 36 and 48 compared to week 24
9. Percentage brain volume change (PBVC) on 3D T1-weighted scans at weeks 12 and 24 compared to baseline (week 0) and weeks 36 and 48 compared to week 24
10. Regional atrophy changes in grey matter (using 3D T1-weighted scans and voxel based morphometry) and core white matter structural changes (using advanced diffusion analysis) at weeks 12 and 24 compared to baseline (week 0) and weeks 36 and 48 compared to week 24.
11. Serial analysis including all time points of magnetisation transfer ratio (MTR) measures of non-enhancing T2 lesions present at baseline (week 0)
12. Serial analysis including all time points of MTR measures of non-enhancing T1 hypointense lesions present at baseline (week 0)
13. Serial analysis including all time points of MTR measures of Gad-enhancing lesions present at baseline (week 0)
14. Serial analysis including all time points of MTR measures of new Gad-enhancing lesions that occur during the trial
15. Serial MTR measures at weeks 0, 12, 24, 36 and 48 of normal appearing grey matter
16. Serial MTR measures at weeks 0, 12, 24, 36 and 48 of normal appearing white matter

17. Serial analysis of diffusion-weighted imaging (DWI) data at all time points to assess axonal integrity and structural changes.

12.8.5. Analysis methods

1. Lesion counts
   (i) The number of new or enlarging T2 lesions will be assessed from PD/T2 fast spin echo images.
   (ii) New T1 hypo-intense lesion measures will be recorded as seen on the post-contrast T1 weighted spin echo scans.
2. Lesion volumes
   A T2 and T1 lesion load calculation will be undertaken by an observer, blinded to the patient details using a semi-automated lesion contouring application programme.
3. Brain atrophy
   (i) Percentage Brain Volume Change (PBVC) will be measured from volumetric T1 weighted scans using the SINA analysis application.
   (ii) Regional atrophy measure using voxel-based morphometry (VBM), which is an unbiased automated technique designed to detect areas of grey matter atrophy independent of the anatomical constraints necessarily associated with *a priori* operator-defined region of interest analysis.
4. MTR
   (i) *Lesion MTR maps* will be determined from the MT scan. White matter lesion masks will be outlined on the MT-off scan and the masks applied to the MTR map to calculate the lesional MTR at each time point. This measure is expected to reflect de- and re-myelination.
   (ii) *GM and NAWM MTR*: As well as calculating the lesional MTR, grey and white matter masks will be segmented and applied to the MTR map to evaluate the mean normal appearing grey and white matter MTR value. Lesion masks will be removed in order to assess the non-lesional tissue.
5. Diffusion
   Diffusion-based measures: Diffusion weighted images allow calculation of indices of structural integrity based on the diffusion coefficient of water molecules and their surrounding environment. These indices can reflect the underlying structural integrity as the hindrance and restriction of water molecules changes with disease.

13. STATISTICS AND DATA ANALYSIS

13.1 RATIONALE

The main aim of each national trial participating in this international collaborative effort, including the present one, is to assess the feasibility, safety and the toxicity of MSC infusion, as administered within this specific treatment protocol, in active MS. To fulfill this aim, all randomised patients receiving at least one MSCs infusion (or infusion media) will be followed for at least two years after the last study drug administration. All adverse events will be recorded and compared in the two treatment groups. Comparative analyses of the incidence of new GEL at MRI acquired on conventional 1.5 T MRI scans over 24 weeks will be conducted as well, to provide preliminary data on the activity of the experimental treatment. However, the main analyses of safety, toxicity and activity will be those on the entire data set obtained by pooling the results of all national trials, for an expected total of 160 randomised patients.

13.2 SAMPLE SIZE

Up to 23 patients are expected to be enrolled in the clinical trial for evaluation of the primary outcome (safety). Data from this clinical trial and from all other national trials will be pooled together for efficacy evaluation which will be evaluated as a decrease in the number of GEL (co-primary outcome).
A sample size of 160 patients was estimated to give an 80% power at a significance level of 5% to detect a decrease of 50% in the number of GEL counted during the 6 months duration of treatment. This number was estimated assuming that the average number of total GEL on 3 MRI scans (scan at weeks 4, 12 and 24) will be 7.4 (SD=11.4), that is half of the number of total number of GEL counted over 6 monthly scans in the phase 2 study of oral fingolimod for MS [43] .

13.3 EVALUATION OF PRIMARY OUTCOME VARIABLES

- Primary outcome: To assess the safety of intravenous therapy with autologous MSCs in MS patients. The safety of MSCs infusion will be evaluated including frequency, timing and severity of any adverse events in both MSCs and placebo treatment groups. A statistical comparison of the number of adverse events between treatment groups will be carried out using a chi-square test.
- Co-Primary outcome: To assess the activity of autologous MSCs in MS patients. The total number of gadolinium-enhancing lesions (GEL) at MRI at week 4, 12 and 24 will be compared between treatment groups using a Negative Binomial (NB) regression analysis, adjusting for the number of GEL at baseline.

13.4 EVALUATION OF SECONDARY OUTCOME VARIABLES

Secondary outcomes: To gather preliminary information about the efficacy of the experimental treatment in terms of combined MRI activity, incidence of relapses and disability progression.

- The total number of GEL lesions at MRI at weeks 4,12 and 24 in the group originally randomised to placebo will be compared with the number of GEL lesions at MRI at weeks 28, 36 and 48 following the infusion using a mixed effect generalised model with NB errors.
- The dependence on time of the total number of GEL at MRI will be analysed in the group originally randomised to active treatment using a mixed effect generalised model with NB errors.
- Combined unique MRI activity (new or enlarging T2, or enhancing or re-enhancing lesions). The total number of new or enlarging T2, or enhancing or re-enhancing lesions at MRI at week 4, 12 and 24 post-treatment will be recorded in the two groups using a Negative Binomial (NB) regression analysis with the treatment group as covariate.
- Number of relapses in MSCs treatment group vs. placebo group will be acquired and using a Negative Binomial (NB) regression analysis with treatment group as covariate
- Time to sustained progression of disability (RRMS group) will be analyzed with a Cox proportional-hazards model
- Proportion of progression-free patients in two groups will be compared with the use of a chi-square test (RRMS patients).
- Mean changes in Multiple Sclerosis Functional Composite (MSFC) score in MSC treatment group vs. placebo group compared to baseline will be analysed with a one-way ANOVA test.

13.5 EVALUATION OF EXPLORATORY OUTCOMES*

Patients will undergo biological 'proof of principle studies' addressing the effect of MSC treatment on immune responses. Immune parameters will be compared within the patients longitudinally and cross-sectionally between post-MSC vs. post-infusion media treatment time points.

* Only for the patients in the first phase of recruitment

13.6 DATA ANALYSIS

The number of GEL counted over 3 MRI scans will be considered a NB variable and analysed using a NB regression model. The model will be adjusted for the number of GEL on the baseline scan and will include treatment group as covariate. The number of relapses during the study period will be analysed with a NB regression model. Time to event variables will be studied with the Cox proportional-hazards model and visualised using the Kaplan-Meier survival curves. Proportion of events in the two treatment groups will be analysed by the chi-square test.
A paired-data analysis will be run to compare the number of GEL during the placebo and during the treatment arm in the group originally randomised to placebo, using both a parametric mixed model with NB errors and a non-parametric Wilcoxon test.

13.7 RECORD RETENTION

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period. If the investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility.

14. MONITORING

14.1 RISK ASSESSMENT

14.1.1. MRI

Magnetic Resonance Imaging (MRI) is a safe and non-invasive method to visualise structure and function *in vivo* of the human brain and body. Earplugs and ear defenders will be provided to attenuate scanner noise, and subjects will be made comfortable with pillows and blankets to prevent lower back pain associated with lying still on the scanner table for up to 90 minutes. All subjects will have a call button should they wish to speak with the radiographer or to stop the scan at any time. At times, atypical findings can present on MRI scanning. Reviewing radiologists will report directly to the treating team if such a finding occurs as per the standard procedures at the MRI centre.

14.1.2. Gadolinium

Gadolinium is a widely used and safe contrast agent in MRI. However, a link has been established between Gadolinium administration and a serious systemic fibrosing disease in patients with

moderate to end-stage kidney disease. Furthermore, Gadolinium is contraindicated in patients with severe renal impairment or those underdoing liver transplantation. Patients will therefore be carefully screened for any unidentified renal impairment – and any patients with serum creatinine clearance < 30 ml/min will be excluded from the study.

14.1.3. Bone marrow sampling
The two most common complications that may occur from this procedure are bleeding and infection at the puncture site. The bleeding at the puncture site is usually controlled with external compression and gauze. Local infection at the puncture site is rare, but may require oral antibiotics. The procedure is performed under sterile conditions to prevent infections and with local anaesthetic to minimise any pain from the procedure.

14.1.4. Lumbar puncture
Common side effects from the lumbar puncture include: (i) Local pain in the lower back: this can occur soon after the procedure and generally settles over 24-48 hours. Local anaesthetic is administered during the procedure to minimise such pain and discomfort. (ii) Headache: 24hrs after the procedure, some people experience a headache that is worse on sitting or standing up. Prolonged headaches, lasting longer than 7 days, develop in only 0.5 - 2 percent of people (about one in 50 - 200 patients). These prolonged headaches usually resolve within 2 weeks of the lumbar puncture.

14.1.5. Skin biopsy*
A punch skin biopsy will be performed. Common risks are bleeding and scarring. Pigmented and oily skins tend to have more scar formation than pale, dry skins. Other complications during and after the procedure are uncommon. Those risks and complications can include, but are not limited to: protracted bleeding, discolouration, cheloid formation, adverse reactions to anaesthesia, infection, pain, and numbness at the biopsy site.

* Only for the patients in the first phase of recruitment

14.1.6. Mesenchymal stem cell infusion
During the infusion, allergic reactions can occur - these include transient fever and headaches. Potentially more significant allergic reactions can occur but are very rare.

14.1.7. Mantoux tuberculin skin test (TST)
Minor reactions such as mild itching, swelling or irritation can occur but do not require treatment. If the patient does not attend for the readout at 48-72 hours, then the test will need to be repeated.

14.2 MONITORING AT THE STUDY COORDINATION CENTRE

Patients registration, randomisation and data entry of all clinical, laboratory and MRI data will be performed via a e-CRF accessible to authorized researchers through the following address: http://ctrials.hsanmartino.it/ist/rnd and http://ctrials.hsanmartino.it/ist/rde. The Centro Clinical Trials will provide the required login and password to each researcher to allow remote registration and randomisation of patients and data entry. Data management will be carried out in compliance with all applicable regulation.

14.3 MONITORING AT LOCAL SITE

This will be performed by the Imperial College Joint Research Compliance Office (JRCO).

15. DOCUMENTATION

15.1 CASE REPORT FORMS

Case Report Forms (CRFs) for individual subjects will be used to record all clinical trial data. They will be completed for every visit and will record patient demographics, informed consent, clinical evaluation at each visit and adverse events. All CRFs will be stored in the subject study binder that will be maintained locally and stored securely. Appropriate coded identification will be used so that subjects will not be identifiable from the CRF.

Data correction within the CRF should be dated, initialled, and explained (if necessary) and should not obscure the original entry i.e. an audit trail should be maintained. Any protocol deviation will be recorded on the form as shown in Appendix 5.

15.2 SOURCE DOCUMENTATION

Source documents including, but not limited to, original signed Informed Consent Forms, prescription forms and laboratory test results will be maintained at the study site in the subject study binder

15.3 SAFETY MONITORING COMMITTEE

A Data Safety and Monitoring Committee (DSMC) will be established to meet at 12 months to review the side-effect profile of both arms of the trial. It is unlikely that a large positive or negative effect from the study medication will be seen, and as such, efficacy data will remain blinded until the end of the study. Data will be presented by the study statistician.

15.4 DATA RETENTION AND ARCHIVING

In compliance with ICH/GCP guidelines and applicable regulatory requirements, the Chief Investigator will be responsible for maintaining all study records and documents pertaining to the conduct of the study. This will include Informed Consent Forms, CRFs and laboratory test results and will be retained as appropriate under current GCP guidelines.

Imperial College London policy is to retain all study documentation for 10 years.

16. REGULATORY ISSUES

16.1 CLINICAL TRIALS AUTHORISATION

This study has Clinical Trials Authorisation from the UK Competent Authority; MHRA. Reference: 19174/0326/001-0001.

16.2 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the Gene Therapy Advisory Committee (GTAC). The Study Coordination Centre will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. Any subsequent amendments to the protocol will require sponsor approval before being submitted to the ethics committee. Once this is obtained, further Trust R&D approval is required before the amendments can be implemented at the site.

16.3 CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

16.4 CONFIDENTIALITY

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. All data arising as part of this study will be treated confidentially, and kept in an anonymised format wherever possible. Personal contact details will be held for communication with the patients participating in the study. Subjects will be assigned a personal study code number which will be used throughout the study and in the analysis of data. The study code will be kept secure in a locked cabinet with only the CI having access. Coded samples or images will also be treated with confidentiality when undergoing analysis. Study data will be kept in a secure database (at the Imperial College Healthcare Trust) entered with the patient's consent. Data will be backed up on secure servers at Imperial College as per standard university procedures. For further analysis, linked anonymised data will be downloaded to local computers. Only linked anonymised data will be available outside of the patient's treating team.

16.5 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies that apply to this study if harm arises either out of design or management of the research. Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study where harm arises to participants in the conduct of the research.

16.6 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

16.7 FUNDING

The UK MS Society and UK Stem Cell Foundation are jointly funding this study. The grant is administered by the MS Society.

16.8 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

17. TRIAL MANAGEMENT

A Trial Management Group will be appointed and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be co-ordinated through the Study Coordination Centre.

Patients registration, randomisation and data entry of all clinical, laboratory and MRI data will be performed via an e-CRF accessible to authorised researchers through the following address: http://ctrials.hsanmartino.it/ist/rnd/ and http://ctrials.hsanmartino.it/ist/rde/. The Centro Clinical Trials, will provide the required login and password to each researcher to allow remote registration and randomisation of patients and data entry. Data management will be carried out in compliance with all applicable regulation.

There will also be a centralised safety committee that will liaise with the local safety monitoring committee on a regular and as-needed basis.

18. PUBLICATION POLICY

The study primary outcomes will be pooled from all studies participating in the International Trial Consortium. All other outcomes and ancillary studies will be analysed and published independently or as seen appropriate by the Investigators. A writing group will be formed by all contributors who played a substantial role in the acquisition, analysis or interpretation of the data and will be chaired by the Principal Investigator. Authorship will be determined on the basis of the specific contribution to the study and to the article write-up. Minor contributions or paid technical work will be acknowledged as appropriate.

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the trial management group and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

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20. APPENDICES

Appendix 1: Kurtzke Expanded Disability Status Scale (EDSS)

The EDSS provides a total score on a scale from 0 to 10. Scores of 1.0 to 4.5 refer to people with a high degree of ambulatory ability and subsequent levels 5.0 to 9.5 refer to loss of ambulatory ability. In addition, it quantifies disability in eight functional systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these.

The functional systems are: (1) Pyramidal (2) Cerebellar (3) Brainstem (4) Sensory (5) Bowel and Bladder (6) Visual and (7) Cerebral or Mental.

The Functional systems are scored on a scale of 0-5 to best reflect the level of disability observed clinically. The 'other' category is not rated numerically.

EDSS scores below 4.0 are determined by the FSS alone. Scores between 4.0 and 9.5 are determined by both gait abilities and the FSS.

0	Normal neurological exam (all grade 0 in Functional Systems (FS); cerebral grade 1 acceptable).
1	No disability, minimal signs in one FS (i.e. one grade 1 excluding cerebral grade 1).
1.5	No disability, minimal signs in more than one FS (more than one grade 1 excluding cerebral grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or 1), or mild disability in three or four FS (three-four FS grade 2, others 0 or 1).
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3 and one or two FS grade 2) or two FS grade 3, others 0 or 1, or five FS grade 2, others 0 or 1.
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters or 0.3 miles.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability. (Usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters or 975 ft).
5.0	Ambulatory without aid or rest for about 200 meters or 650 ft; disability severe enough to impair full daily activities (e.g., to work full day without special provisions). (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
5.5	Ambulatory without aid or rest for about 100 meters or 325 ft; disability severe enough to impair full daily activities. (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
6.0	Intermittent or constant unilateral assistance (cane, crutch, or brace) required to walk about 100 meters or 325 ft. with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)
6.5	Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters or 65 ft. (Usual FS equivalents are combinations with more than two FS grade 3+.)
7.0	Unable to walk beyond about 5 meters or 16 ft. even with aid, essentially restricted to wheelchair, wheels self in standard wheelchair a full day and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone.)
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfers, wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+.)
8.0	Essentially restricted to bed or chair or perambulated in wheelchair; but may be out of bed much of the day; retains may self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
8.5	Essentially restricted to bed for much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
9.0	Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4.)
9.5	Totally helpless bed patient; unable to communicate or effectively eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+.)
10	Death due to MS.

Appendix 2: Multiple Sclerosis Impact Scale (MSIS-29)

The MSIS-29 is a questionnaire whereby the patient indicates the impact of MS on their daily life over the past two weeks by placing a circle around the answer that best represents their situation.

	In the past 2 weeks how much has your MS limited your ability to	Not at all	A little	Moderately	Quite a bit	Extremely
1	Do physically demanding tasks?	1	2	3	4	5
2	Grip things tightly (e.g. turning on taps)?	1	2	3	4	5
3	Carry things?	1	2	3	4	5
	In the past 2 weeks how much have you been bothered by	Not at all	A little	Moderately	Quite a bit	Extremely
4	Problems with your balance?	1	2	3	4	5
5	Difficulties moving about indoors?	1	2	3	4	5
6	Being clumsy?	1	2	3	4	5
7	Stiffness?	1	2	3	4	5
8	Heavy arms and/or legs?	1	2	3	4	5
9	Tremor of your arms or legs?	1	2	3	4	5
10	Spasms in your limbs?	1	2	3	4	5
11	Your body not doing what you want it to?	1	2	3	4	5
12	Having to depend on others to do things for you?	1	2	3	4	5
	Please check that you have answered all the questions above before going onto the next section.					
	In the past 2 weeks how much have you been bothered by	Not at all	A little	Moderately	Quite a bit	Extremely
13	Limitations in your social and leisure activities at home?	1	2	3	4	5
14	Being stuck at home more than you would like to be?	1	2	3	4	5
15	Difficulties using your hands in everyday tasks?	1	2	3	4	5
16	Having to cut down the amount of time you spent on work or other daily activities?	1	2	3	4	5
17	Problems using transport (e.g. car, bus, train, taxi etc)?	1	2	3	4	5
18	Taking longer to do things?	1	2	3	4	5
19	Difficulty doing things spontaneously (e.g. going out on the spur of the moment)?	1	2	3	4	5
20	Needing to go to the toilet urgently?	1	2	3	4	5
21	Feeling unwell?	1	2	3	4	5
22	Problems sleeping?	1	2	3	4	5
23	Feeling mentally fatigued?	1	2	3	4	5
24	Worries related to your MS?	1	2	3	4	5
25	Feeling anxious or tense?	1	2	3	4	5
26	Feeling irritable, impatient or short tempered?	1	2	3	4	5
27	Problems concentrating?	1	2	3	4	5
28	Lack of confidence	1	2	3	4	5
29	Feeling depressed?	1	2	3	4	5

Please check that you have circled **ONE** number for each question only.

Appendix 3: Multiple Sclerosis Function Composite Score (MSFC)

This is a series of tests including:

- The Timed 25 – Foot Walk
- The 9 - Hole Peg Test
- The Paced Auditory Serial Addition Test (PASAT)

The Timed 25 – Foot Walk (T25-FW)

The T25-FW is a quantitative mobility and leg function performance test based on a timed 25-foot walk. It is the first component of the MSFC to be administered at each visit. The patient is directed to one end of a clearly marked 25-foot course and is instructed to walk 25 feet as quickly as possible, but safely. The time is calculated from the initiation of the instruction to start and ends when the patient has reached the 25 feet mark. The task is immediately administered again by having the patient walk back the same distance. Patients may use an assistive device when performing this task.

The score for the T25-FW is the average of the two completed trials. This score can either be used individually or as part of the MSFC composite score.

The 9-Hole Peg Test (9-HPT)

The 9-Hole Peg Test (9-HPT) is a brief, standardised quantitative test of upper extremity function. Reliability and validity have been assessed and normative data is available. Both the dominant and non-dominant hands must be tested twice.

The patient should be seated at a table with a small shallow container holding nine pegs and a wood or plastic block containing nine empty holes. The patient is instructed to pick up the nine pegs, place them in the holes and then remove them as quickly as possible (one at a time), to replace them in the original shallow container. The total time to complete the task is recorded using a stopwatch.

The final score for the 9-HPT is the average of the four trials. The two trials with each hand are averaged, converted to the reciprocals of the mean times for each hand and then the two reciprocals are averaged. This score can be used individually or as part of the MSFC composite score.

Paced Auditory Serial Addition Test (PASAT)

This is a measure of cognitive function that assesses auditory information processing speed and flexibility as well as calculation ability. The PASAT is presented using an audio cassette tape or compact disc to ensure standardisation in the rate of stimulus presentation. Single digits are presented every 3 seconds and the patient must add each new digit to the immediately prior to it. (*Shorter inter-stimulus intervals e.g. every 2 seconds or less have been used with PASAT but tend to increase the difficulty of the test*).
The score for the PASAT is the total number correct out of 60 possible answers. This score can be used individually or as part of the MSFC composite score.

Appendix 4: SF-36 Assessment (Version 2)

THE FOLOWING QUESTIONS ASK FOR YOUR VIEWS ABOUT YOUR HEALTH AND HOW YOU FEEL ABOUT LIFE IN GENERAL. IF YOU ARE UNSURE ABOUT HOW TO ANSWER ANY QUESTION, TRY AND THINK ABOUT YOUR OVERALL HEALTH AND GIVE THE BEST ANSWER YOU CAN. DO NOT SPEND TOO MUCH TIME ANSWERING, AS YOUR IMMEDIATE RESPONSE IS LIKELY TO BE THE MOST ACCURATE ANSWER.

PLEASE TICK (✓) THE ANSWER THAT BEST REPRESENTS YOUR RESPONSE.

1. **In general, how would you say your health is?**

   - ☐ Excellent
   - ☐ Very Good
   - ☐ Good
   - ☐ Fair
   - ☐ Poor

2. **Compared to 3 months ago, how would you rate your health in general now?**

   - ☐ Much better than 3 months ago
   - ☐ Somewhat better now than 3 months ago
   - ☐ About the same as 3 months ago
   - ☐ Somewhat worse now than 3 months ago
   - ☐ Much worse now than 3 months ago

3. **The following items are about activities you might do during a typical day. Does your health limit you in these activities? If so, how much?**

   a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   b. Moderate activities, such as moving table, pushing a vacuum cleaner, bowling or playing golf.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   c. Lifting or carrying groceries.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   d. Climbing several flights of stairs.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   e. Climbing one flight of stairs.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   f. Bending, kneeling and stooping.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   g. Walking more than one mile.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

   h. Walking several blocks.
   - ☐ Yes, limited a lot
   - ☐ Yes, limited a little
   - ☐ No, not limited at all

SF-36 Assessment (Version 2) continued **Page 2 of 5**

i. Walking one block.
- ☐ Yes, limited a lot
- ☐ Yes, limited a little
- ☐ No, not limited at all

j. Bathing or dressing yourself.
- ☐ Yes, limited a lot
- ☐ Yes, limited a little
- ☐ No, not limited at all

4. **During the past 2 weeks, what proportion of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

a. Cut down the amount of time you spent on work or other activities.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

b. Accomplished less than you would like.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

c. Were limited in the kind of work or other activities.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

d. Had difficulty performing the work or any other activity (for example, it took more effort)
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

5. **During the past 2 weeks, what proportion of the time have you had any of the following problems with your work or other regular daily activities as a result of emotional problems (such as feeling depressed or anxious)?**

a. Cut down the amount of time you spent on work or other activities.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

b. Accomplished less than you would like.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

c. Didn't do work or other activities as carefully as usual.
- ☐ **All of the time**
- ☐ **Most of the time**
- ☐ **Some of the time**
- ☐ **A little of the time**
- ☐ **None of the time**

SF-36 Assessment (Version 2) continued Page 3 of 5

6. **During the past 2 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?**
   - ☐ Not at all
   - ☐ Slightly
   - ☐ Moderately
   - ☐ Quite a bit
   - ☐ Extremely

7. **How much bodily pain have you had during the past 2 weeks?**
   - ☐ None
   - ☐ Very Mild
   - ☐ Mild
   - ☐ Moderate
   - ☐ Severe
   - ☐ Very Severe

8. **During the past 2 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**
   - ☐ Not at all
   - ☐ Slightly
   - ☐ Moderately
   - ☐ Quite a bit
   - ☐ Extremely

9. **These questions are about how you feel and how things have been with you during the past 2 weeks. For each question, give the answer that comes closest to the way you have been feeling. How much of the time during the past 2 weeks?**

   a. Did you feel "full of life"?
   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ None of the time

   b. Have you been a very nervous person?
   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ None of the time

   c. Have you felt so "down in the dumps" nothing could cheer you up?
   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ None of the time

   d. Have you felt calm and peaceful?
   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ None of the time

   e. Did you have a lot of energy?
   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time

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- ☐ A little of the time
- ☐ None of the time

f. Have you felt downhearted and blue?

- ☐ All of the time
- ☐ Most of the time
- ☐ A good bit of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

g. Did you feel worn out?

- ☐ All of the time
- ☐ Most of the time
- ☐ A good bit of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

h. Have you been a happy person?

- ☐ All of the time
- ☐ Most of the time
- ☐ A good bit of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

i. Did you feel tired?

- ☐ All of the time
- ☐ Most of the time
- ☐ A good bit of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

10. During the <u>past 2 weeks</u>, how much of the time <u>your physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc.)?

- ☐ All of the time
- ☐ Most of the time
- ☐ A good bit of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

11. How *TRUE* or *FALSE* is <u>each</u> of the following statements for you?

a. I seem to get sick a little easier than other people

- ☐ Definitely true
- ☐ Mostly true
- ☐ Not Sure
- ☐ Mostly false
- ☐ Definitely false

b. I am as healthy as anybody I know

- ☐ Definitely true
- ☐ Mostly true
- ☐ Not Sure
- ☐ Mostly false
- ☐ Definitely false

c. I expect my health to get worse

- ☐ Definitely true
- ☐ Mostly true
- ☐ Not Sure

SF-36 Assessment (Version 2) continued **Page 5 of 5**

- Mostly false
- Definitely false

d. My health is excellent

- Definitely true
- Mostly true
- Not Sure
- Mostly false
- Definitely false

----------THANK YOU FOR COMPLETING THE QUESTIONNAIRE----------

Appendix 5: Protocol deviation form

PROTOCOL DEVIATION FORM	
TRIAL TITLE: Stem cells in Rapidly Evolving Active Multiple Sclerosis **PHASE:** II	**PATIENT INITIALS:** **SCREENING NUMBER:** **CLINICAL TRIAL NUMBER:**
REASON FOR DEVIATION	
ASSESSMENT OF DEVIATION 1. **Major deviation** ☐ 2. **Minor deviation** ☐	**APPROVED BY** **Name** **Signature** **Date** Principal Investigator/Sub-investigator

Appendix 6: Declaration of Helsinki

Recommendations guiding medical doctors in biomedical research involving human subjects.
Adopted by the 18th World Medical Assembly, Helsinki, Finland 1964 amended by the 29th World Medical Assembly, Tokyo, Japan 1975 and the 35th World Medical Assembly, Venice, Italy, October 1983 and the 41st World Medical Assembly, Hong Kong, September 1989.

INTRODUCTION
It is the mission of the medical doctor to safeguard the health of people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, 'The health of my patient will be my first consideration', and the international code of medical Ethics declares that, 'A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient'

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic, and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice, most diagnostic, therapeutic, or prophylactic procedures involve hazards. This applies especially to biomedical research. Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research that may affect the environment. In addition, the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of such experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the Investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of this objective is in a proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to out weigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely given informed consent, preferably in writing.
10. When obtaining informed consent for the research project, the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of his official relationship.
11 In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with the national legislation. Where physical form or mental incapacity makes it impossible to obtain informed consent or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with the national legislation.
    Whenever the minor child is in fact able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.
12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present declaration are complied with.

II Medical research combined with professional care (clinical research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, re-establishing health or alleviating suffering.
2. The potential benefits, hazards and discomforts of a new measure should be weighed against the advantages of the best diagnostic and therapeutic methods.
3. In any medical study, every patient including those of a control group, if any, should be

assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.
5. If the physician considers it essential not to obtain informed consent the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (1.2.)
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III Non-therapeutic biomedical research involving human subjects (non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers – either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The Investigator or the Investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subjects.

Appendix 7: Stem cell referrals standard operating procedure

MULTIPLE SCLEROSIS TRIALS COLLABORATION

STANDARD OPERATING PROCEDURE FOR MANAGING STEM CELL REFERRALS

AUTHOR: DAVID WILKIE

17 October 11
VERSION: 1.0

BACKGROUND

The following system has been devised to help manage the influx of referrals from different channels enquiring about stem cell research, as follows:

Methods of Referral:

- Healthcare Professionals
- Professional Bodies e.g. MS Society/Trust
- Self-referrals (Patient)
- Friends or Relatives
- Overseas enquiries
- Other

Via the following Channels:

- Written (post)
- E-mail
- Phone
- Voicemail

Following a number of meetings with the study team, it was agreed that one point of reference for all incoming referrals was the most appropriate way to proceed. Thus, the following e-mail address was created:

stemcellms@imperial.ac.uk

Dr Paolo Muraro, as Principal Investigator, had previously responded to a selection of patients up to a known time point, but not those enquiring beyond this. For this reason, the first task was to organise correspondence and split these into two time periods and by methods of referral.

All referrals were input into an electronic database for future reference including the following information if known:

1. Name of person making contact (if other than the patient)
2. Patient's name (Surname, First name)

3. Mailing address
4. Date of first enquiry
5. Tel no 1
6. Tel no 2
7. Email address
8. GP/Consultant information
9. How did they hear of trial?
10. MS details
11. Method
12. Response Date

The team decided on two separate auto-responses to send to patients outlining the basic entry criteria for the trial as follows:

The first aimed at those who had received a previous response via MS Society:

Thank you for your interest in the stem cell study that was recently covered in the media. Following the press release in the media on 28th July 2011, we received a very high number of phone calls, letters and e-mails from interested subjects. It has been difficult to manage such a massive flow of enquiries and soon after the initial response we felt we could not consider additional subjects for potential participation. After consulting the MS Society, on 1st August we issued a statement that the study had identified enough participants.
We have now put in place a study management team and we would like to give everyone who contacted us more information about the study and the opportunity for them to know if they would meet the study basic entry criteria and, if so, to reconfirm their interest in participating.

Summary of the basic entry criteria:

1) Confirmed diagnosis of MS - Relapsing-Remitting, Secondary Progressive or Primary Progressive MS
2) Age 18-50 (inclusive)
3) Disease duration between 2 and 10 years (inclusive)
4) Ability to walk at least 20 metres with bilateral assistance - without resting

There will be additional entry criteria and the participation in the study is restricted to those eligible for treatment under the NHS and who reside in the UK for the duration of the study.
The study has yet to receive ethical approval. Until this occurs, we will only register your interest at this stage.
If you feel you are interested and meet the basic criteria, please e-mail stemcellms@imperial.ac.uk and write "CONFIRMATION OF INTEREST" into the subject field.

This is an experimental treatment and a safety study and thus there is no guarantee that it will have any benefit to participants. For this reason we do not recommend any delay in treatment that has been offered to you.
We are unable to reply to individual questions but we will release more information to those that have confirmed interest as soon as it becomes available.

Sincerely,
On behalf of the study team
Dr Paolo Muraro
Clinical Reader in Neuroimmunology
Honorary Consultant Neurologist
Centre for Neurosciences
Division of Experimental Medicine
Department of Medicine, Imperial College

The second to all new enquiries:

Thank you for your interest in the stem cell study that was recently covered in the media.
We have received a high number of enquiries and for this reason, we would like to apologise for the delay in responding.
We are unable to reply to individual questions but we will aim to release information on the study in the hope that this will answer your queries.
The study has yet to receive ethical approval. Until this occurs, we will only register interest at this stage and enclose a summary of the basic entry criteria:

1) Confirmed diagnosis of MS - Relapsing-Remitting, Secondary Progressive or Primary Progressive MS
2) Age 18-50 (inclusive)
3) Disease duration between 2 and 10 years (inclusive)
4) Ability to walk at least 20 metres with bilateral assistance - without resting

There will be additional entry criteria and the participation in the study is restricted to those eligible for treatment under the NHS and who reside in the UK for the duration of the study.

If you feel you are interested and meet the basic criteria, please e-mail stemcellms@imperial.ac.uk and write "CONFIRMATION OF INTEREST" into the subject field.

This is an experimental treatment and a safety study and thus there is no guarantee that it will have any benefit to participants. For this reason we do not recommend any delay in treatment that has been offered to you.
We will release more information to those that have confirmed interest as soon as it becomes available.

Sincerely,
On behalf of the study team
Dr Paolo Muraro
Clinical Reader in Neuroimmunology
Honorary Consultant Neurologist
Centre for Neurosciences
Division of Experimental Medicine
Department of Medicine, Imperial College

Managing On-going and new referrals

NB: All patient information should be recorded in the electronic database and a written record stored in the 'Stem Cell Referrals' folder. The response date and method should also be recorded.

- All e-mail enquiries should be responded to with the appropriate e-mail auto-response (as above).
- All patients who send written correspondence should be responded to with the appropriate auto-response (as above) on Imperial headed paper.
- All phone enquiries should be responded to and the person enquiring advised to e-mail stemcellms@imperial.ac.uk to register interest.
- If an e-mail is ambiguous in content and clarification is needed then the best approach is to alert Paolo Muraro who will respond accordingly. Please keep a record of any correspondence that is forwarded on for follow-up.
- It may also be necessary for Paolo Muraro to respond to e-mails from healthcare professionals or professional bodies. Again, he should be alerted to this. In such instances, the autoresponse may not be the most appropriate response.

Confirmation of Interest

If a patient confirms their interest in the trial (irrespective of their eligibility), their interest should still be recorded in the 'confirmation of interest' section of the electronic database.
A paper copy of the confirmation e-mail should also be filed – especially if new information has been added.

Electronic Database

This encompasses all referrals to date. It is especially important to keep a log of phone referrals as a written record does not exist and to manage any updates to existing data.

When any updates are made to the database, the file name should be updated to reflect the date of change for eg. 'Stem Cell Patient Referrals 17Oct11' and saved to an encrypted hard drive – ideally the X Drive setup especially for the MS Service and accessible only by David Wilkie, Richard Nicholas and Paolo's Muraro's team.

'Stem Cell Referrals' folder.

- Correspondence is ordered A-Z.

- It is ordered with priority given to patient's surnames but when this is not known, the referrer's surname should be used.
- When a patient's details have been entered into the electronic database, a corresponding print off of the e-mail should be filed.
- When correspondence has been entered on to the electronic database, a tick should be placed in the top right hand corner of the correspondence to acknowledge that this has occurred – so as to avoid double-entry.

Stem Cell MS E-mail Management

- When an e-mail has been printed and/or recorded in the electronic database, the e-mail should be tagged green and can be archived if necessary.
- The tag can be achieved by right clicking on the appropriate message with Outlook and selecting 'categorize' then 'Green category'.
- Ideally the e-mails should be checked on a daily basis to determine if any messages require urgent follow-up.

Appendix 8: MRI Patient Information Sheet

Version 1.0 14 May 2012

MRI Patient Information Sheet

What is a MRI?
MRI stands for Magnetic Resonance Imaging, a screening technique used to visualise the soft tissue inside your body.

How does MRI work?
MRI combines a powerful magnet with radio-frequency pulses. These collect signals that are then processed by a sophisticated computer to form pictures of your brain.

What is MRI used for?
MRI gives highly detailed pictures of the brain. Doctors can use MRI scans to see if there are any changes to a patient's brain over time.

What do I need to do before my MRI scan?
No special preparation is needed for your scan and you may continue to eat and drink as normal and take any prescribed medicines.

It is helpful if you can come for your scan in clothing without metal parts, such as hooks or zips. If this is not possible, then you will need to change into a hospital gown. We would also request that you leave any valuable possessions at home.

Before your scan, you will need to complete a safety questionnaire. Due to the powerful **magnet** that is used in MRI, not all patients can be scanned – such as those with **surgical clips**, **metal hip replacements, a pacemaker or metal fragments in the eyes.**
If you are concerned in any way, please contact our team before your scan appointment on telephone number 020 8383 0675

What will happen during your scan?
For your scan, you will lie down on a scanning bed that will slowly move into a small tunnel. You will experience no discomfort or pain, but may hear quite loud mechanical sounds from the scanner. Ear plugs will be provided to minimise any discomfort from these sounds. If you suffer from claustrophobia (fear of small places) you may not be able to have an MRI scan.

In order to obtain good quality pictures of your brain, it is important that you do not move during the scanning procedure. Special supports will be used to help keep your head still. The radiographer operating the scanner can both see and hear you throughout the procedure so if you have any concerns, you only have to say. You can expect to be in the scanner for up to 90 minutes. The scans will then be sent electronically to the scan centre to be analysed over the course of the clinical study. Only those staff involved in the clinical study will be allowed to see the scans.

Appendix 9: Informed consent for MRI Scan

Version 1.0 14 May 2012

Informed Consent for MRI Scans

		Patient's initials
1.	I have read and understood the patient information sheet relating to the MRI scans.	☐
2.	I have had the opportunity to ask questions regarding the procedures and have obtained answers to my satisfaction.	☐
3.	I have completed and understood the safety questionnaire.	☐
4.	I confirm that I give my consent to undergo MRI scans.	☐

Please print and sign your name below and add today's date.

.....	.....	.....
Name of patient	Signature of patient	Date
.....	.....	.....
Name of person Taking consent	Signature of person taking consent	Date