Promoting and measuring remyelination and neuroprotection in clinical trials of people with multiple sclerosis

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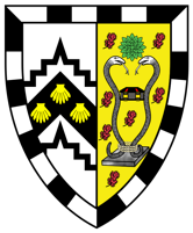

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This thesis is submitted for the degree of Doctor of Philosophy

Personal declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text.

It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text.

It does not exceed the prescribed 60,000-word limit for the degree committee for the Faculties of Clinical Medicine and Veterinary Medicine.

Nick Cunniffe

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Abstract

The most tractable strategy to delay or prevent the progressive phase of MS is to promote endogenous remyelination; doing so restores nerve conduction and prevents demyelinated axons from degenerating. The rate-limiting stage in this process is differentiation of oligodendrocyte progenitor cells (OPCs) into mature, myelinating, oligodendrocytes. In animals, agonism of the retinoid X receptor (RXR)-γ enhances remyelination in this way. Metformin also promotes remyelination, this time by overcoming an age-associated block to the responsiveness of OPCs to pro-differentiation factors.

In this PhD I show that bexarotene, a non-selective agonist of the RXR receptor, promotes remyelination in people with relapsing remitting multiple sclerosis. Converging evidence from electrophysiology and neuroimaging in a phase II clinical trial (CCMR One) demonstrate that this occurs in demyelinated lesions and is greatest in lesions located in grey matter regions of the brain. I additionally conducted analyses which suggest an age-dependency of bexarotene's remyelinating effect and led a follow-up sub study of the trial participants which showed the treatment effect afforded by bexarotene is sustained, years after treatment. Unfortunately, bexarotene was poorly tolerated, and so the legacy of this trial will likely be one of shaping the framework for future assessments of remyelinating therapies. As such, I designed, obtained funding and secured approvals for a clinical trial to test the remyelinating effect of the combination of metformin with clemastine (CCMR Two), implementing lessons from CCMR One in the trial design; this is due to commence participant recruitment in 2021.

In exploring treatments for progressive forms of MS, not limited to the process of remyelination, I worked with the MS Society to develop and implement a rigorous, expert-led, evidence-based approach to the selection of licensed drugs for repurposing and testing in clinical trials of people with progressive MS. I reviewed the preclinical and clinical literature for a list of compounds and condensed these into a database of summary documents. These were presented to a panel of experts and people affected by MS, ultimately leading to four treatments being recommended for immediate testing in progressive MS trials: R-α-lipoic acid, metformin, the combination treatment of R-α-lipoic acid and metformin, and niacin.

Regrettably, much of my research has been halted by the COVID-19 pandemic. In this PhD I had additionally sought to evaluate electrophysiological techniques for quantifying remyelination and neuroprotection – multifocal VEP and saccadometry – in cohorts of people with MS and in the setting of the CCMR Two trial. Instead, when these studies were delayed, I embraced an opportunity to help the Cambridge COVID-19 research effort, working on the RECOVERY trial in the first wave, and leading my own project to analyse enrolment to treatment trials, ultimately describing the barriers to, and implications of, low recruitment rates in advance of the subsequent waves. Consequently, further exploratory research with electrophysiology and the CCMR Two trial will be the subject of post-doctoral research.

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Contents

Publications arising from PhD

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Foreword

I am interested in finding treatments for people with multiple sclerosis. There have been three overarching aims of my PhD. First, to advance knowledge in remyelination research by translating preclinical results into clinical trials of people with MS. Second to understand and develop the electrophysiological and imaging outcome measures required to demonstrate their effects in phase II trials. And third, to evaluate other treatments for progressive MS, not limited to the strategy of enhancing endogenous remyelination.

In my first chapter, I provide an introduction to the bulk of the thesis, starting with a brief examination of the aetiology, pathology, diagnosis, phenotype and current management of MS, before describing the biology of remyelination, why this fails in MS and how this might be therapeutically targeted. I then summarise the remyelinating therapies that have been, and are being, evaluated and discuss the best approach to measure remyelination in people. This will lead into the key research questions for the PhD.

In organising this thesis, I have apportioned each research project a chapter, with the methods, results and discussions contained within each. I start by presenting the Cambridge Centre for Myelin Repair trials: the results of CCMR One, related work I have undertaken to investigate the age-dependency and durability of remyelination in response to bexarotene, and the development of CCMR Two. I then describe the processes by which we selected licensed drugs for repurposing in a clinical trial of people with progressive MS. My final chapter details the key conclusions from the thesis alongside a presentation of my future research plans.

Impact of the COVID-19 pandemic

Unfortunately, my PhD has been significantly impacted by the coronavirus disease 2019 (COVID-19) pandemic. A primary objective of my PhD was to translate the recent discovery of the remyelinating effect of metformin in rats, into a clinical trial of people with MS. Despite securing funding for this trial in October 2019, CCMR Two has still not been able to start. Additionally, over the course of the first year of the PhD I rekindled an affinity for electrophysiology, in particular the use of visual evoked potentials (VEPs) and saccadometry, having recognised the importance of the former as a sensitive marker of remyelination across a range of trials. Indeed, as part of this PhD I spent 3 weeks at Sydney University with Prof Sasha Klistorner and built a collaboration with his research group to bring multifocal VEP to our trials in Cambridge. Regrettably, my natural history electrophysiology projects were halted in March 2020, not long after their ethical approvals, and I have not been able to recruit any patients since that time as they – as observational studies – were deemed level 3 research in the NIHR recovery strategy. Helpfully, we were able to demonstrate that our follow-up sub study of the Cambridge CCMR One participants qualified as level 2 (a non-COVID trial that might benefit patients), and I present these results in Chapter 4.

I have embraced opportunities to help the research response to the pandemic. I am particularly proud to have contributed to getting the RECOVERY trial up and running in Cambridge within just 10 days, and to have worked to successfully recruit patients during the first wave. I also led a research study, under the supervision of Dr Mark Toshner, to better understand the recruitment barriers we had been observing in the first wave and the potential effects this might have on the national response – these data are presented in the penultimate chapter.

I therefore leave my PhD with more research questions than when I first started, but I hope to have opportunities to answer these over the next few years of my research career.

Chapter 1: Introduction

Overview

Multiple Sclerosis (MS) is a chronic, primarily inflammatory, disorder of the central nervous system (CNS) characterised by focal lymphocytic infiltration causing damage to myelin and axons.[1-3] It is estimated to effect over 130,000 people in the United Kingdom[4] and is the leading cause of non-traumatic disability in young adults in the developed world.[5,6] Typical clinical features include weakness, sensory loss, diplopia, reduced visual acuity, dysarthria, dysphagia, ataxia, and bladder dysfunction; largely a reflection of the distribution of demyelinating foci throughout the CNS.[7] In 85% of patients, there is an initial period of episodic neurological dysfunction followed by partial or complete recovery (relapsing remitting MS, RRMS).[8] Over time, the clinical picture often develops to one of progressive disability (secondary progressive MS, SPMS),[9] while in 15% the illness is progressive from the outset (primary progressive MS, PPMS).[10]

There now exists an extensive therapeutic armamentarium against the inflammation of MS.[11] These disease modifying treatments (DMTs) reduce the incidence and severity of new lesions by limiting the activity and availability of immune cells, manifesting clinically through reductions in relapse rates and disability accrual.[12-21] However, the “therapeutic window” for treatment with these immunotherapies is limited;[22] best long-term results on disability are seen if an anti-inflammatory treatment is started within five years of the first clinical episode of demyelination in relapsing remitting disease.[23] Furthermore, while 16 DMTs are currently licensed for RRMS, only one – ocrelizumab – is approved for primary progressive disease,[24] and even then its effects are so modest that several reimbursement agencies, notably NICE in the UK, declared it only cost-effective in a subset of people with new or contrast-enhancing lesions on MRI and a disease duration of less than 15 years. Similarly, while siponimod confers a small reduction in disability progression to those with active SPMS,[25] it is approved by NICE only if the patient is still having relapses

or has imaging evidence of inflammatory activity. Evidently, the degenerative mechanisms that characterise progressive MS are not sufficiently targeted by immunomodulatory compounds[26] and the greatest unmet need for people with progressive forms of MS are effective neuroprotective and neurorestorative treatments.[27]

The promotion of regeneration of the myelin sheath, through enhancing the process of endogenous remyelination, has emerged as one of the most tractable prospects to delay, prevent or reverse progression.[28] This is grounded in experimental evidence that demonstrates that the myelin sheath (and its associated oligodendrocytes) does not just facilitate nerve conduction but is also directly protective against degeneration.[29-34] In this introduction, I describe the aetiology, pathology, diagnosis, and current management of MS, before describing the biology of remyelination, why this fails in MS and how this might be therapeutically targeted. I then summarise the remyelinating therapies that have been, and are being, evaluated and discuss the best approach to measure remyelination in people, which is proving increasingly essential in order to translate promising preclinical research into clinical trials.

Aetiology of multiple sclerosis

The causation of multiple sclerosis is multifaceted, but inevitably the result of interactions between genetic, epigenetic and environmental risk factors. Some of these, particularly vitamin D deficiency, smoking and obesity, are potentially modifiable prior to the development of MS, but also impact the disease course and efficacy of different treatments.[35]

Obesity

Elevated body mass index (BMI) in young adults increases the risk of developing MS across a range of observational studies.[36-39] For example, in the nurses' health study, women with a BMI ≥30 at age 18 had a relative risk of developing MS of 2.25 (95% CI 1.50, 3.37).[38] In a mendelian randomization analysis, the genetic factors that influence BMI similarly increase the odds of MS, strengthening BMI's position as an MS risk factor, outside of its potential confounding factors.[40] Meanwhile, speaking to an ongoing contribution of adiposity to the course of disease in MS, hyperlipidaemia increases relapse rates (HR 1.67, 95% CI 1.07, 2.61),[41] disease activity,[42] and disability progression.[43] BMI also affects paraclinical measures of progression, being negatively correlated with grey matter volume and brain parenchymal fraction in people with RRMS and clinically isolated syndrome,[44] and negatively correlated with the ganglion cell inner plexiform layer (GC-IPL) at optical coherence tomography;[45] though these were both uncontrolled studies and, given elevated BMI has been associated with brain volume loss in the general population,[46] a disease-specific effect beyond this trend has not been demonstrated. Mechanistically, there is evidence that obesity has pro-inflammatory effects in a myriad of autoimmune diseases,[47] and perhaps most pertinently for MS, it directs macrophages toward a pro-inflammatory M1 polarization,[48] while adiposity-related factors, such as leptin, promote a pro-inflammatory T_H1 profile.[49]

Vitamin D

The primary source for vitamin D is from UV exposure, but it is also available from dietary sources: it is converted to $25(OH)D_3$ (which gives a measure of vitamin D

status) and then to 1,25$(OH)_2D_3$ (the biologically active form). Prospective studies of 25$(OH)D_3$ collectively show a 50-60% reduced risk of MS with levels ≥75 nmol/L, and a 2-fold increased risk of MS with levels <30 nmol/L.[50-52] Meanwhile, higher 25$(OH)D_3$ is predictive of reduced relapse rates, fewer new active lesions, and reduced T2 lesion volume.[53] Alternative explanations for these associations include other immunosuppressive effects of sunlight (outside of the vitamin D pathway)[54] or of other nutrients that tend to be grouped with dietary vitamin D (e.g. polyunsaturated fatty acids).[55] However, akin to BMI, mendelian randomisation studies do support an independent role of vitamin D.[56] The correction of vitamin D insufficiency has therefore been posited as a strategy to prevent multiple sclerosis,[57] yet limitations in observational studies restrict the extent to which such inverse associations can be attributed specifically to vitamin D.[58] Additionally, vitamin D supplementation has, thus far, not led to improved clinical outcomes in trials of people with MS.[59,60] Conclusive testing in large prevention and treatment trials is required, but issues such as sample size, suitable populations, and appropriate dosing of vitamin D are challenges to their design.

Cigarette smoking

The increased susceptibility risk afforded by smoking tobacco increases with intensity and duration[61-63] and is greater in men than in women.[64] Smoking also interacts with some of the strongest associated risk genes (such as the presence of HLA-DRB1*15:01 and absence of HLA-A*02), suggesting that a priming of the immune response in the lungs may lead to MS in genetically susceptible individuals.[65] Similar results have been seen in those exposed to organic solvents.[66] Smoking is additionally associated with a worse disease course,[67] with the development of antibodies to interferon-β-1a and natalizumab;[68,69] while smoking cessation has been shown to delay the conversion to SPMS.[70]

Epstein-Barr virus

The notion that MS might be a late complication of a viral infection in genetically susceptible individuals has long been speculated and, while a causative virus has never been found, a link to EBV seems likely. Nearly all people with MS are

seropositive for antibodies to EBV nuclear antigen 1 (EBNA1) or its viral capsid antigen (VCA),[71] almost all EBNA1-negative individuals serologically convert prior to MS onset,[72] and the relative risk of MS conferred by a history of infectious mononucleosis in a meta-analysis of 19,390 patients was 2.17 (95% CI 1.97, 2.39).[73] Synergy has been shown with the presence of HLA-DRB1*15:01;[74] molecular mimicry is a likely candidate mechanism.

Genetic susceptibility

The increased heritability within families and increased risk with degree of relatedness provided early evidence that genetic factors are implicated in the development of MS.[75,76] Comparisons of risk between relatives (segregation analysis) has generally suggested a polygenic model in which risk is determined by a single moderate-effect allele (OR 3-4) and many smaller-effect alleles (OR <1.5), with interactions between such risk alleles accounting for much of the apparent heritability of the disease.[77] Genome-wide association studies have now characterised more than 200 gene variants that increase the risk of the disease, revealing a significant overrepresentation of immunologically relevant genes;[78] a finding consistent with the assertion that autoimmune mechanisms are central to the development of MS (discussed below).[2] It is noteworthy that not one gene implicated in the susceptibility to MS has a clear link to oligodendrocytes or myelination; while one of the most strongly associated SNPs occurred in a region associated with the galactosylceramidase (GALC) gene[79] – the product of which is an essential component of myelin – this is in tight linkage disequilibrium with G protein coupled receptor 65 (GPCR65), itself involved in T cell apoptosis.[80] The main risk allele for MS, HLA-DRB*15:01, confers an odds ratio of 3.10.[79] However, this is present in 13.3% of the UK population, while fewer than 0.3% of people carrying it develop MS, and significant numbers without the allele also develop MS.[77] Thus, genetic risk factors are not in themselves sufficient to cause MS, contributing in the region of a third of the overall MS disease risk.[81]

The immunopathogenesis of multiple sclerosis

MS is characterised by imbalanced interactions between effector and regulatory subpopulations of immune cells.[82] The aforementioned genome wide association studies,[78,83] supported by recent work with MS-discordant monozygotic twins,[84] highlights a genetic predisposition largely centred on immune pathways involving T and B cells, ultimately resulting in autoreactive cells that are capable of infiltrating and promoting damage within the CNS.[85] However, CNS-resident cells, particularly microglia, are also implicated in genetic risk.[86]

How autoreactive T cells become activated in the periphery is still a matter of debate. One option is molecular mimicry (e.g. in the context of systemic infection), in which T cells against non-self epitopes cross-react with self-myelin epitopes.[87-89] Another is through recognition of CNS sequestered antigens that are released into the periphery as neo-antigen (either by drainage to the lymph nodes or through carriage by antigen presenting cells).[90] Whatever the inciting event, the result is a cycle of tissue damage yielding the generation of pathogenic CD4+ T helper 1 (T_H1) cells and T_H17 cells, which enter the circulation, translocate to the CNS, and exert their effector functions.[3] Of course, in health, peripheral tolerance mechanisms would be expected keep such autoreactive cells at bay: in MS, reduced regulatory T cell function or resistance of effector T/B-cells to suppressive mechanisms leads to a breakdown of self-tolerance.[91]

In MS, there is a predilection for inflammatory cell infiltrates to accumulate in certain regions: around the lateral ventricles and corpus callosum, in the juxtacortical areas, cortex, optic nerves and brainstem, and throughout the spinal cord.[1,92-94] In early disease, there is little damage outside of these so-called MS lesions. CD8+ T cells predominate in a perivascular inflammatory infiltrate, while macrophages, B cells and plasma cells can also be found.[95] The particular immunologic patterns of demyelination in active lesions are heterogenous.[96] The most common types (patterns I and II) show an activated macrophage/microglial inflammatory background, with a perivascular and parenchymal infiltration of T cells; pattern II is

additionally distinguished by immunoglobulin and complement deposition. Less commonly, in about 25% of biopsied lesions, oligodendrocyte apoptosis predominates on an inflammatory background (pattern III).[96]

What determines the long-term fate of a given lesion – whether inflammation resolves or smoulders and whether regeneration takes place – is not well understood.[2] Longitudinal imaging studies suggest that lesions forming in younger people repair more effectively,[97] a finding in line with evidence that ageing is associated with a declining ability of the immune-system to clear myelin debris and of oligodendrocyte progenitors to facilitate remyelination.[98-100] (The myriad reasons for remyelination failure will be considered in a subsequent section.) In chronically demyelinated lesions, denuded axons remain vulnerable and degenerate: leading candidate mechanisms include energy deficiency on account of mitochondrial oxidative stress[101,102] and loss of ionic homeostasis following ion channel redistribution.[103-105] Additional damage occurs in those lesions with persistent inflammation: a smouldering lesion is formed, characterised by a slowly expanding rim of activated macrophages/microglia surrounding an inactive centre.[106]

As MS progresses, a more diffuse inflammatory T cell and B cell infiltrate, coupled with widespread microglial and astrocyte activation is seen; inflammation becoming organised in the CNS and proceeding in the absence of continued immune cell infiltration from the periphery.[85] Very few breaches seem to exist in the blood-brain barrier by this point,[107,108] leading to a compartmentalised inflammation driven by a diffuse microglial inflammation which seemingly drives expansion of demyelinated lesions.[109] There is also increasing cortical involvement, particularly at subpial regions, which is associated with the formation of lymphoid follicles in the meninges.[110]

The phenotype and diagnosis of multiple sclerosis

The advent of effective treatments for MS has reinforced the need for making an early yet secure diagnosis. In the majority, clinical features indicate involvement of motor, sensory, visual and autonomic systems, nonetheless many other symptoms and signs may be present. Unfortunately, none are specific to MS, though Lhermitte's symptom (neck flexion resulting in an electric-shock sensation running down the spine and limbs) and Uhthoff's phenomenon (the worsening of symptoms and signs with increasing body temperature, for example during a hot bath) are particularly characteristic.[1] Diagnosis therefore requires the synthesis of clinical, imaging and laboratory findings, to demonstrate dissemination in space and time, while excluding other neurological conditions (Table 1.1).[111]

The McDonald diagnostic criteria were developed in 2001[112] and have been increasingly refined over the course of revisions in 2005,[113] 2010,[114] and 2017.[115] They are particularly helpful in the setting of a single episode when the diagnosis of multiple sclerosis is suspected, typically termed a clinically isolated syndrome (CIS), by defining the clinical and paraclinical findings required to fulfil dissemination in time (DIT) and dissemination in space (DIS). The MRI criteria were established in their current format in 2010, such that DIS can be demonstrated with a T2 lesion in at least 2 out of 4 locations characteristic of MS (juxtacortical, periventricular, brainstem, and spinal cord), while DIT can be exhibited by the presence of new T2 lesions on serial imaging, or the co-occurrence of gadolinium-enhancing and non-enhancing lesions.[114] Now, following the most recent 2017 revisions of the McDonald criteria, the presence of CSF-specific oligoclonal bands can establish DIT, and (if detected) cortical lesions at MRI may be also used to demonstrate DIS (Table 1.2).[115]

	Neurological features	Investigation findings
Neuromyelitis optica spectrum disorder (NMOSD)	Concurrent optic neuritis and transverse myelitis; intractable hiccough or nausea/vomiting	Longitudinally extensive (>3 vertebral segments) cord lesion, chiasmal involvement. Serum antibodies to AQP-4 and MOG. CSF OCB infrequent
Acute disseminated encephalomyelitis (ADEM)	Multifocal symptoms typical, often monophasic, encephalopathy common. Often in context of antecedent viral illness	Spectrum of inflammatory demyelination on MRI from small lesions to large tumefactive lesions with mass effect. Can impact any region of the CNS. CSF pleocytosis
Neurosarcoidosis	Meningitis, myelopathy, cranial nerve involvement (VII and II primarily), raised intracranial pressure	Meningeal enhancement. Brain white matter lesions. Raised serum and CSF ACE. CSF OCB sometimes present
CNS vasculitis	Confusion, headache, personality change, stroke-like presentations, seizures	Multiple ischaemic lesions, predominant at cortico-subcortical junction, intracranial haemorrhage, microbleeds. Serum ANCA raised. CSF OCB sometimes present
Susac's syndrome	Visual loss, sensorineural hearing loss, headache, memory loss, encephalopathy	Snowball lesions in corpus callosum, leptomeningeal enhancement, focal and small WM and GM lesions. CSF OCB infrequent
Connective tissue disorders (SLE, Sjögren's syndrome, antiphospholipid syndrome)	Neuropsychiatric symptoms, seizures, ischaemic episodes	Brain infarcts and haemorrhage, venous sinus thrombosis. Subcortical and cord lesions. Positive ANA, anti-SS-A, anti-SS-B and anti-Sm
Neuro-Behçet's disease	Brainstem syndrome, myelopathy	Large brainstem lesions, subcortical and spinal cord lesions, venous sinus thrombosis. CSF pleocytosis. HLA-B51

Table 1.1. Non-exhaustive differential diagnosis of multiple sclerosis. AQP-4, aquaporin-4; MOG, myelin oligodendrocyte glycoprotein; CSF, cerebrospinal fluid; OCB, oligoclonal bands; ACE, angiotensin converting enzyme; ANCA, anti-neutrophil cytoplasmic antibody; WM, white matter; GM, grey matter; SLE, systemic lupus erythematosus; ANA, anti-nuclear antibody; HLA, human leukocyte antigen.

Number of clinical attacks	Number of lesions with objective clinical evidence	Additional findings required for a diagnosis of MS
≥2	≥2	None
≥2	1 (as well as clear historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
≥2	1	DIS evidenced by additional clinical attack implicating a different CNS site, or by MRI
1	≥2	DIT evidenced by additional clinical attack or by MRI OR demonstration of CSF-specific oligoclonal bands
1	1	DIS evidenced by an additional clinical attack or MRI implicating a different CNS site AND DIT evidenced by an additional clinical attack OR new lesion by MRI OR demonstration of CSF-specific oligoclonal bands

Table 1.2. The 2017 McDonald criteria for the diagnosis of multiple sclerosis. CNS, central nervous system; DIS, dissemination in space; DIT, dissemination in time.

The diagnosis of primary progressive MS is similarly established in the McDonald criteria.[115] This requires at least one year of progression in disability independent of clinical relapses, plus the presence of 2 of: (i) one or more T2-hyperintense lesions in areas characteristic of multiple sclerosis in the brain, (ii) two or more T2-hyperintense lesions, and (iii) the presence of CSF-specific oligoclonal bands.

The diagnosis of secondary progressive MS is, however, more challenging. In most clinical contexts, SPMS is defined by a history of gradual accumulation of disability after an initial relapsing remitting course, yet there remain no universally accepted criteria to determine the point at which one transitions from RRMS to SPMS.[116] Instead, the diagnosis of SPMS is often made in retrospect, with the benefit of years of gradual progression. One study reported that, on average, a transition period of three years elapsed between the possibility of SPMS being first entertained to the diagnosis being definitively made.[117] Disentangling the residual effects of relapses

and disability progression adds a further layer of complexity; relapses are not uncommon in SPMS, occurring in 12% and 24% of those with SPMS allocated to placebo over 2 years of follow-up in two recent clinical trials.[118,119] In an effort to establish an objective SPMS definition that is predictive of long-term disability outcomes, a recent study compared 576 different definitions of SPMS across 17,356 patients, and found the best performance required 3 months of confirmed disability progression in the absence of relapse (if the EDSS step was 5.5 or less, an increase of 1.0 or more was required; if the EDSS was 6.0 or above, an increase of 0.5 was needed), requiring an eventual EDSS step of ≥4.0 and pyramidal score of ≥2.[120] Experimental medicine trials, meanwhile, have typically required two years of progression for participant inclusion.[118,119,121]

Yet, from the perspective of selecting treatments, a perhaps more biologically important distinction for people with progressive MS is to characterise whether the illness is active – that is to say, have evidence of new focal inflammation as determined by clinical relapses and/or development of new lesions at MRI – and whether it is progressing in the absence of inflammation, as recognised in the 2013 consensus criteria.[116]

The management of multiple sclerosis

Management of acute relapses

A relapse is defined by patient-reported symptoms or objectively observed signs that are typical of an acute inflammatory demyelinating event in the central nervous system, with a duration of at least 24 hours, in the absence of fever or infection.[115] The latter point is particularly important, as fluctuations in symptoms can occur for reasons other than a relapse, for example due to fatigue, fever, infection, and heat (often termed a pseudo-relapse). Clinical guidelines therefore encourage physicians to first rule out infection (particularly of the urinary and respiratory tract) before diagnosing relapse and contemplating treatment.[122]

The mainstay of treatment is with corticosteroids. These curtail the duration of the relapse,[123] but are widely stated to have no effect on the extent of the recovery that is eventually made.[124-126] Taken together with the potential side effect profile of even short courses of steroids – including, but not limited to, insomnia, dysphoria, hyperglycaemia, gastrointestinal distress, osteonecrosis of the femoral head, and cataracts – it is widespread practice to reserve courses of steroids for those relapses that are particularly disabling. Helpfully, oral administration of high-dose methylprednisolone is not inferior to intravenous administration,[127] and so relapses do not typically require hospitalisation. Steroid-refractory relapses meanwhile, can be treated with plasmapheresis.[128] Indeed, as might be expected, patients exhibiting lesions with a predominance of immunoglobulin deposition (immunopathological pattern II), are the most likely to respond to therapeutic plasma exchange.[129] However, as with corticosteroids, there is no clear evidence that this impacts long term functional recovery.

Management of acute optic neuritis

The discussion of relapse management would not be complete without consideration of acute optic neuritis (AON). Prospective trials have demonstrated a reproducible

benefit of high-dose corticosteroids, perhaps most notably with the optic neuritis treatment trial (ONTT)[130] in which 457 participants were randomised within 8 days of AON to receive either placebo, oral prednisolone (1 mg/kg/day for 14 days), or intravenous methylprednisolone (250 mg 4 times daily for 3 days, followed by prednisolone 1 mg/kg/day for 11 days). The rate of visual recovery over the first 15 days was greater in the intravenous methylprednisolone group, while contrast sensitivity and colour vision (though not visual acuity) were improved at 6 months.[130] However, by 12 months, there was no difference in visual function, compared to placebo;[131] the conclusion that high-dose corticosteroids hasten recovery, but do not change long term functional outcome, has been reinforced by subsequent meta-analyses.[132]

Meanwhile, the ONTT also reported an unexpected result: oral (intermediate dose) prednisolone increased the risk of recurrent optic neuritis compared to both intravenous methylprednisolone and placebo.[130] While this particular conclusion has been disputed,[133] taken together with an evident lack of efficacy over placebo, only high-dose corticosteroids are now recommended for AON. More recent studies have established that oral high-dose methylprednisolone is not demonstrably inferior to intravenous high-dose methylprednisolone in the treatment of AON,[134,135] thereby negating the cost and tolerability concerns of repeated hospital attendances to receive IV steroids.

So, alike MS relapses affecting other locations in the nervous system, high-dose corticosteroids seemingly hasten the speed, but not the extent, of functional recovery. Yet, the definition of recovery needs to extend beyond high-contrast visual acuity; in the ONTT, >90% of participants, regardless of treatment group, recovered to, what the authors described as, a “normal” visual acuity of 20/50 by 6 months, creating an erroneous impression that most make an excellent recovery following AON.[130] However, a follow-up study 5-8 years later, found persistent abnormalities of affected eyes compared to fellow eyes in contrast sensitivity (58% vs 17%), visual fields (33% vs 12%), colour vision (37% vs 18%) and visual acuity (39% vs 16%).[136] Similarly, electrophysiological and structural techniques such as visual evoked

potentials and optical coherence tomography reveal persistent demyelination and neuroaxonal loss as sequelae of AON.[137] Thus, the recovery from AON is not complete, and can therefore provide a unique model for evaluating treatment response to drugs capable of promoting remyelination and preventing neurodegeneration.[138]

Treatment of relapsing remitting multiple sclerosis

The last 25 years have seen an expanding repertoire of anti-inflammatory disease modifying treatments (DMTs) deployed in the treatment of relapsing remitting multiple sclerosis (RRMS) and clinically isolated syndrome (CIS). There are currently nine DMT classes approved for use in RRMS: glatiramer acetate, β interferons, dimethyl fumarate, sphingosine 1-phosphate receptor modulators (fingolimod and siponimod), teriflunomide, cladribine, natalizumab, alemtuzumab and B-cell targeted monoclonal antibodies (ocrelizumab and rituximab). With contrasting mechanisms of action, efficacy and safety, weighing treatment decisions has become increasingly complex for patients and clinicians alike.[3]

Injectable therapies (Table 1.3)

The injectables were the earliest type of DMT to be approved for the treatment of RRMS; the first interferon-β preparations in 1993 and glatiramer acetate in 1996 (Table 1.3). In clinical trials, these drugs reduced the annualised relapse rate (aRR) by in the region of 30% compared to placebo, and modestly prolonged the time to an increase in disability.[12,13,139,140] They were also of similar efficacy in comparative trials.[141,142] Since the introduction of these drugs, new preparations have followed that have allowed a reduction in the frequency of administration.[143,144] An additional benefit of these drugs is the rarity with which any serious adverse events – such as hepatitis and pulmonary hypertension[145,146] – occur. However, tolerability, particularly injection-site and flu-like symptoms, means compliance can be variable.[12,13,139,140]

DMT	Mechanism of action	Evidence of efficacy	Monitoring	Safety (pregnancy)
Glatiramer acetate (Copaxone, Brabio) 20 mg daily or 40 mg three times weekly, SC	Random amino acid copolymer believed to inhibit MBP-reactive T cells and probably stimulate regulatory T cells[147]	29% relapse rate reduction vs placebo[13]	Initiation: none Monitoring: none	Injection site and post-injection reactions. Lipoatrophy. (No reported harms in pregnancy or breast feeding)
Interferon-β SC IFNβ-1b (Betaferon, Betaseron, Extavia) IM IFNβ-1a (Avonex) SC IFNβ-1a (Rebif) Variable dosing: three times weekly, weekly or alternate weeks	Enhancement of regulatory T cell activity, reduction in pro-inflammatory cytokine production, and inhibition of lymphocyte trafficking into CNS[147]	27-34% reduction in relapse rate[12,139,140,144]	Initiation: FBC, UEs, LFTs, immunoglobulins Monitoring: FBC, UEs, LFTs, blood pressure, TSH, interferon binding antibodies	Injection site and flu-like reactions. Rarely causes bone marrow suppression, liver failure and thrombotic microangiopathy (manifest as hypertension and renal impairment). (Probably safe in pregnancy and with breast feeding[148])

Table 1.3. The injectable disease modifying treatments. MBP, myelin basic protein; SC, subcutaneous; IFN, interferon; IM, intramuscular; CNS, central nervous system; FBC, full blood count; UEs, urea and electrolytes; LFTs, liver function tests; TSH, thyroid stimulating hormone.

Monoclonal antibodies (Table 1.4)

After 10 years of using injectables alone, the inception of natalizumab, a humanised antibody against α-4 integrin on the surface of lymphocytes capable of reducing their translocation into the CNS,[149] resulted in a substantial step up in terms of efficacy. The AFFIRM trial showed a reduction of the aRR by 68% and of sustained disability progression by 42% compared to placebo.[18] Unfortunately, this improvement in efficacy is counterbalanced by the risk of progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the CNS by the JC virus,[150] which carries a 23% mortality rate. The major risk factors for PML are a positive anti-JCV status, prior immunosuppressant use, and a long treatment duration with natalizumab; anti-JCV antibody index and duration of natalizumab treatment can be used to estimate risk.[151] An additional concern is rebound of disease activity upon treatment discontinuation.[152,153] It is therefore more usually reserved for those with rapidly evolving severe MS: typically requiring 2 clinically disabling relapses in the last year and, on imaging, one or more gadolinium-enhancing lesions or an increasing T2 lesion load.

Another highly effective treatment for MS is alemtuzumab, a humanized monoclonal antibody directed against CD52, which causes lymphopenia followed by homeostatic reconstitution resulting in a prolonged alteration of the immune response.[154] It was first trialled in patients with progressive MS, where it did not impact clinical disability, but did significantly reduce the formation of new MRI lesions.[155] Ensuing studies in RRMS showed reductions in relapse rates of 91-94% compared to placebo[156,157] and of 49-55% compared to interferon-β;[19,20] taken together, this experience led to the hypothesis that there is a 'therapeutic window' whereby immunotherapies are beneficial if started early in the disease.[22] Additionally, the benefits of alemtuzumab were shown to be durable, with efficacy maintained for over 5 years in >70% of patients who had received two courses of alemtuzumab.[158] Adverse events of alemtuzumab treatment include infusion reactions on account of cytokine release syndrome (which are treated with steroids and anti-histamines), and herpetic infections (that can be countered by prophylactic acyclovir); human papilloma virus (HPV) infection (with the potential corollary of cervical dysplasia) is an additional

consideration, though unpublished data from England and Wales shows no difference from the population mean (personal communication). Secondary autoimmunity is also a consequence, usually occurring 2-3 years after treatment, which includes thyroid disorders (in >30%), thrombocytopenia (in 1-3%) and glomerulonephritis (in <1%).[154] Unfortunately, since regulatory approval, there have been reports of other severe adverse events including listeriosis,[159] haemolytic anaemia with necrotizing leukoencephalopathy,[160] alveolar haemorrhage,[161] hemophagocytic lymphohistiocytosis[162] and stroke.[163] This triggered a review of alemtuzumab by the European Medicines Agency in 2019, and its use has since been refined to those with rapidly evolving severe MS (as above), with no concurrent autoimmune disease, and those suffering relapses despite other DMTs.

Finally, positive results from clinical trials of antibodies to CD20, which deplete mature B cell pools, suggests that these have a central role in MS pathogenesis. While rituxumab was subject of a positive phase II study,[164] it is ocrelizumab that has come through phase III trials of RRMS, reducing the aRR by 47% and disability progression by 40% compared to IFNβ -1a.[21] This is also the only drug to have shown evidence of efficacy in PPMS (discussed below).[24]

DMT	Mechanism of action	Evidence of efficacy	Monitoring	Safety (pregnancy)
Natalizumab (Tysabri) Infusion 300 mg every 4-6 weeks	Antagonist of the α-4 integrin component of the very late antigen 4 adhesion molecule on lymphocytes, preventing crossing of the BBB	68% reduction in relapse rate and 42% reduction in disability progression compared to placebo[18]	Initiation: FBC, LFTs, UEs, TFT, JCV serology, VZV, HIV, HBV, HCV, MRI-B Monitoring: FBC, LFTs, JCV serology (unless already known high titre), MRI scan at least annually – but as often as 3-monthly for high-titre patients	Hypersensitivity reactions, opportunistic infections (HSV, VZV, PML), and rebound disease activity upon treatment cessation. (Not known to be harmful to foetus, but treatment typically suspended at 34 weeks and restarted soon after birth.)
Alemtuzumab (Lemtrada) Infusion First course: 12 mg/day for 5 days. Additional courses: 12 mg/day for 3 days.	Anti-CD52 humanised monoclonal antibody, resulting in lymphopenia followed by immune reconstitution	49-55% reduction in relapse rate compared to interferon[19,20]	Initiation: FBC, UEs, LFTs, TFTs, TB, HBV, VZV, urinalysis, MRI, cervical smear Monitoring: FBC, UEs, TFTs, urinalysis	Opportunistic infections (herpes, varicella, listeria), autoimmunity (thyroid disorders, ITP, nephropathy), leucopenia and lymphopenia. Rare associations with HLH, stroke, haemolytic anaemia, alveolar haemorrhage and sarcoidosis. (Pregnancy can be contemplated 4 months after last dose.)

Ocrelizumab (Ocrevus) Infusion: 600 mg, 6-monthly	Anti-CD20 humanised monoclonal antibody, depletes B cells	47% reduction in relapse rate compared to interferon[21]	Initiation: FBC, HBV, VZV Monitoring: FBC	Infusion reactions, herpes infection, potential link to PML, and possible increased risk of malignancy (particularly breast) (Pregnancy not advised for 12 months after last dose.)

Table 1.4. Monoclonal antibody treatments of multiple sclerosis. BBB, blood-brain barrier; FBC, full blood count; LFTs, liver function tests; UEs, urea and electrolytes; TFT, thyroid function tests; JCV, JC Virus; VZV, varicella zoster virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; PML, progressive multifocal leukoencephalopathy; TFTs, thyroid function tests; TB, tuberculosis; ITP, immune thrombocytopenic purpura; HLH, hemophagocytic lymphohistiocytosis.

Oral treatments (Table 1.5)

Fingolimod, the first licensed oral treatment for RRMS, is a relatively non-selective sphingosine-1-phosphate (S1P)-receptor modulator that prevents lymphocyte egress from lymph nodes. Its efficacy has been shown across several trials with reductions of relapse rates of 48-55% compared to placebo[16,165] and by superiority to intramuscular interferon-β.[166] It is, however, associated with an increased risk of infections (in particular herpes zoster, but also PML), deranged LFTs, basal cell cancer, and macular oedema. It can also cause bradycardia and so participants are typically observed for 6-12 hours after the first dose. In the UK, fingolimod can be prescribed if patients are still having relapses after taking one of the interferons, glatiramer acetate, or dimethyl fumarate. It can also be prescribed to a patient previously on natalizumab whom has a high risk of PML. Some of the safety concerns are expected to be addressed by ozanimod, a selective modulator of S1P1 and S1P5 receptors.[167]

Teriflunomide was the second oral treatment to be licensed for RRMS: it is a metabolite of leflunomide that inhibits proliferation of B and T cells and, while it has comparable efficacy to interferon, it has a more significant adverse event profile including diarrhoea, nausea, hair thinning, increased levels of liver enzymes (in 7%) and teratogenicity.[14,168-170] It is therefore not commonly prescribed in the UK as other oral treatments and injectables are usually preferred.

Dimethyl fumarate (DMF) is the most commonly prescribed oral MS drug. It has many effects. In particular, it induces a shift in the cytokine profile of T helper (T_H) cells from pro-inflammatory (T_H1) to an anti-inflammatory (T_H2) profile,[171] and it regulates redox balance in monocytes and T cells through activation the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.[172] Over two phase III trials, DMF led to a reduction in the aRR by 44-53% and reduced the risk of disability progression over 2 years by 35% compared to placebo.[15,173] No increased risk of malignancy or serious infection has been observed but PML has occurred, usually in the setting of lymphopenia ($<0.5 \times 10^9$/L).

Cladribine is the most recently added oral option for RRMS. It is a prodrug, converted by intracellular phosphorylation to the active purine analogue, 2-chlorodeoxyadenosine triphosphate, which disrupts cell metabolism and inhibits DNA synthesis and repair in lymphocytes.[174] In a phase III study of 1,326 people with RRMS this led to a reduction in relapse rate of 58% and a lower rate of sustained disability progression compared to placebo.[17]

DMT	Mechanism of action	Evidence of efficacy	Monitoring	Safety (pregnancy)
Cladribine (Mavenclad) 2 courses of treatment over 2 years	(Prodrug of) purine analogue which depletes B and T lymphocytes	58% reduction in relapse rate compared to placebo[17]	Initiation: FBC, HBV, HIV Monitoring: FBC	Herpes zoster infections, lymphopenia, rash, alopecia. (Teratogenic and 6 months wash out required.)
Fingolimod (Gilenya) 0.5 mg orally once per day	Prevents autoreactive lymphocytes from leaving lymph nodes	48-55% reduction in relapse rate compared to placebo[16,175]	Initiation: ECG, OCT, dermatologic review, FBC, LFTs, VZV, BP Monitoring: FBC, LFTs, OCT at 4 months, annual skin check, BP	First dose bradycardia, macular oedema, herpes zoster, deranged LFTs, hypertension, basal cell carcinoma and, rarely, PML and herpes encephalitis. (Risk of congenital malformations[176] and requires >2 months washout before conception.)
Dimethyl fumarate (Tecfidera) 240 mg orally twice per day	Nrf2 pathway activation and NFkB inhibition causing alteration in immune cell activation and inflammatory cytokine balance	44-53% reduction in relapse rate compared to placebo[15,173]	Initiation and monitoring: FBC, LFTs, UEs	Flushing and gastrointestinal disturbance. Causes lymphopenia and (rarely) PML. (Limited data about safety in pregnancy. Typically recommend to switch if wishing to become pregnant.)

Teriflunomide (Aubagio) 14 mg orally once daily	Inhibits B and T cell proliferation by inhibiting a mitochondrial enzyme involved in pyrimidine synthesis	31-36% relapse rate reduction vs placebo.[14,168,169]. Comparable to interferon[170]	Initiation: FBC, LFTs, BP Monitoring: FBC and LFTs (2-weekly for 6 months, then 2-monthly). BP	Nausea, diarrhoea, hair thinning, deranged LFTs. (Patients should not get pregnant for 2 years after stopping treatment. If fall pregnant then should undergo accelerated elimination with activated charcoal and cholestyramine.)

Table 1.5. Oral DMTs used in the treatment of multiple sclerosis. ECG, electrocardiogram; OCT, optical coherence tomography; FBC, full blood count; LFTs, liver function tests; VZV, varicella zoster virus; BP, blood pressure; PML, progressive multifocal leukoencephalopathy; UEs, urea and electrolytes; HIV, human immunodeficiency virus; HBV, hepatitis B virus.

The long-term effects of disease modifying treatments

The pivotal studies that demonstrated the efficacy of disease modifying treatments have largely focussed on reductions in relapse rates and disability progression, often alongside MRI measures of disease activity, over short duration clinical trials.[177] However, while it seemed intuitive that these treatments would be beneficial in the long term, evidence for this is more recent, perhaps reflective of the challenges to conducting and controlling observational cohort studies over extended time periods.

An early approach was to interrogate potential links between DMT exposure and mortality. For example, Goodin et. al. performed long term follow up of participants from the pivotal interferon β-1b randomised clinical trial;[178] they showed that after a median follow up of 21.1 years, all-cause mortality was significantly lower in those that had been treated with IFNβ-1b, when compared to those that had been treated with placebo (HR 0.532 p=0.017, 95% CI 0.314, 0.902). Similar results were

returned in a study of the effects of β-interferon in a population cohort: increased survival was observed in those who had been treated with β-interferon for ≥3 years when compared to matched controls (HR 0.44; 95% CI 0.30, 0.66).[179]

The demonstration that DMTs are effective in preventing long term disability progression has been similarly challenging. Cohort studies have reported a declining rate of conversion from relapsing MS to secondary progressive MS compared to earlier natural history studies, and posited that this is representative of progress following the advent of DMTs.[180] Yet, robust evidence that DMTs impact disability progression has been a recent development.[23] Utilising a real-world, prospective, observational cohort study – the MS Base database – Brown and colleagues made a series of important observations. First, that treatment significantly lowered the probability of secondary progression compared to untreated controls: for interferon-β or glatiramer acetate (HR 0.71 p<0.001 95% CI 0.61, 0.81), for fingolimod (HR 0.37 p<0.001 95% CI 0.22, 0.62), for natalizumab (HR 0.61 p = 0.005 95% CI 0.43, 0.86) and for alemtuzumab (HR 0.52 p=0.009 95% CI 0.32, 0.85). Second, that the probability of conversion to secondary progressive MS was lower if treated within 5 years of disease onset (for interferon-β and glatiramer acetate, HR 0.77 p=0.03 95% CI 0.61, 0.98). And third, that patients initially receiving fingolimod, alemtuzumab, or natalizumab had a significantly lower risk of converting to secondary progressive MS than matched controls who were initially treated with the (lower efficacy) glatiramer acetate or interferon-β (HR 0.66 p=0.046 95% CI 0.44, 0.99).[23]

Treatment decisions in relapsing remitting multiple sclerosis

Following a first clinical attack of multiple sclerosis (a clinically isolated syndrome), there is evidence, from randomised trials and open label extension studies, that first-line therapies might reduce the conversion to clinically definite MS.[181,182] However, the 2010 and 2017 revisions of the McDonald diagnostic criteria have changed the conditions for these diagnoses;[114,115] the latest revisions now allow the earlier diagnosis of RRMS, and DMTs are not recommended outside of satisfying these criteria. Whether or not to start treatment following recognition of radiologically

isolated syndrome has similarly been controversial.[183] First line therapies are presently considered in the setting of 2 relapses in the past 2 years, 1 relapse in the past 2 years with radiological activity, or in rapidly evolving severe MS.[184]

A particular distinction in first-line treatment strategies is whether to advocate an escalation strategy (initially commencing moderately efficacious DMTs, which have generally good safety profiles, and escalating to higher efficacy DMTs in the circumstance of breakthrough disease activity (clinical relapse and/or new lesions at MRI)) or whether to deploy an induction strategy (choosing high-efficacy drugs from the outset).[185] Population level data has indicated that early intensive treatment is superior to moderate efficacy DMTs in reducing the accumulation of disability,[23,186] but this is yet to be demonstrated in a comparative trial. The DELIVER-MS study (NCT03535298), which will test the effects of these contrasting strategies on brain volume loss and disability, seems likely to be informative in shaping these different treatment approaches. Of course, particular attention will be needed to account for the safety considerations of these approaches when strategizing treatment approaches, which has not yet been done in any systematic way.

Ultimately, with no biomarker currently able to identify the optimum treatment for a particular patient at a particular timepoint, the decision of when and how to treat a patient is reliant on patient characteristics and preferences.[187] Treatment algorithms have been developed to help guide appropriately evidence-based selections based on such patient characteristics,[184,188] and consensus statements made on important considerations such as pregnancy.[189] Clearly, eliciting patient preferences must be optimised when balancing the potential benefits against side effect profiles and the burden of adherence to safety monitoring.[190]

Treatments for progressive forms of multiple sclerosis

The expanding repertoire of anti-inflammatory disease modifying treatments for RRMS contrasts with a paucity of effective therapies for the 15% of people that present with progressive disability (primary progressive MS; PPMS), and indeed the

80% of RRMS patients who develop progression after RRMS (secondary progressive MS; SPMS);[191] most immunotherapies have failed in non-active progressive disease.[192]

With regard to SPMS, the first positive randomised placebo-controlled phase III trial was for interferon β-1b, which significantly delayed time to 12-week confirmed disability progression (OR 0.65 p=0.0008; 95% CI 0.52, 0.83), reduced relapse rate (IFN-β-1b 0.44 vs placebo 0.64, p=0.002) and reduced the proportion of patients with active scans (p=0.0046),[193] though it did not influence brain atrophy.[194] The IMPACT trial of intramuscular interferon β-1a similarly met its primary endpoint: the multiple sclerosis functional composite (MSFC) of the 25-foot walk, paced auditory serial addition test (PASAT) and 9-hole peg test (9HPT);[195] a result driven by an effect on the 9HPT. Unfortunately, these effects were not replicated in subsequent studies;[196,197] it seems likely, given the higher relapse rates in the former (positive) trials, that interferon-β has an impact on relapse-related disability only. Consequently, current NICE guidance advises the use of interferon-β among people with SPMS if they are continuing to have relapses. Natalizumab has also been the subject of intensive investigation in SPMS. ASCEND was a phase III, randomized, double-blind, placebo-controlled trial, which showed no between-group difference in a multicomponent measure of disability progression (formed of the EDSS, timed walk and 9HPT).[198] Natalizumab-treated SPMS patients did have reduced 9HPT progression however, which led the authors to conclude that Tysabri reduced progression in upper limb function and might, over a duration longer than 2 years, improve ambulation. Yet, the study that seems most likely to change practice in SPMS is the EXPAND trial of the sphingosine 1-phosphate receptor antagonist, siponimod.[25] This met its primary endpoint, demonstrating a reduction in time to 12-week confirmed disability progression (HR 0.79 p=0.013 95% CI 0.66, 0.95), alongside other positive endpoints including percentage brain volume change (p=0.0002). This has not yet been demonstrated to be superior to interferon β-1b, but has now been licensed in the US and EU, as well as being approved by NICE, though only for those SPMS patients with relapses or imaging features suggestive of inflammatory activity.

For PPMS, the results of trials of immunotherapies have been more clearly and consistently negative.[199,200] The only positive phase III trial has been the ORATORIO trial in which ocrelizumab reduced 12-week disability progression by 6.4% compared to placebo (HR 0.76; 95% CI 0.59 – 0.98),[24] though it is noteworthy that all participants in this trial were ambulant, the mean age of the population was 44 years, all had a disease duration of ≤15 years, and 28% of patients on the study drug had gadolinium enhancement at baseline. Consequently, these results are not generalisable to the entire population of people with PPMS, and this is reflected in NICE's approval which requires patients be ambulant up to 20 metres, have had symptoms for ≤15 years and evidence of MS activity at MRI. From the phase II perspective, another notable result for an anti-inflammatory agent has been the SPRINT MS trial of ibudilast, which led to significant reductions in brain parenchymal fraction loss compared to placebo ($p = 0.04$).[201]

In contrast, neuroprotective and remyelinating therapies may have a wider window of opportunity than immunotherapies and address the pathogenic mechanisms of progressive MS not controlled by DMTs.[202] Several phase II trials have been completed, but we are yet to see robust evidence of efficacy of such a drug in a phase III trial.[192] While remyelinating therapies will be discussed in more detail in the next section, one of the most promising agents currently at this stage is simvastatin. In the MS-STAT trial, 140 SPMS patients were randomised 1:1 to receive simvastatin 80 mg or placebo, which yielded a 43% reduction in mean annualized rate of brain atrophy ($p = 0.003$).[118] MS-STAT2 is currently testing this in 1180 SPMS patients in a phase III study (NCT03387670). Similarly high hopes had been had for amiloride, fluoxetine and riluzole, but when tested in the MS-secondary progressive multi-arm randomisation trial (MS SMART) study in 445 people with SPMS, no treatment effect on brain atrophy (percentage brain volume change) was seen over 2 years.[119] However, one of the legacies of this trial will be its demonstration of the feasibility and efficiency of multi-arm trials, in particular in the testing of repurposed drugs.[192] Indeed, preparative work for this adaptive trial is the rationale for my work with the MS Society's treatment selection group, discussed in Chapter 6.

Remyelination in multiple sclerosis

The roles of myelin and oligodendrocytes

Myelination offers a far better way of increasing the conduction velocity of nerve fibres than simply increasing axon size. Myelin increases the transverse, insulating, resistance of the axon membrane while the voltage-gated sodium and potassium channels are virtually confined to short unmyelinated nodes of Ranvier. The action potential is therefore propagated by the comparatively rapid and energy efficient process of saltatory conduction.[203] It follows that loss of myelin leads to slower transmission of the action potential, and hence prolonged latency, but can also lead to conduction block.[204] Remyelination is therefore a way to restore saltatory conduction,[205] and clinical function,[206] after demyelination.

Additionally, oligodendrocytes directly support the neuron, for example through providing lactate for metabolism and generation of ATP.[30-32] Pathological studies and animal models also suggest that axonal degeneration is reduced in remyelinated areas.[29,33,34] Taken together, a remyelinating therapy has the potential to restore function and prevent neurodegeneration in multiple sclerosis.

Meanwhile, it is increasingly apparent that myelin regulation is a dynamic process in which both newly formed oligodendrocytes and pre-existing oligodendrocytes remodel myelin, often in response to activity, to facilitate learning and plasticity.[207] Whether activity-dependent remyelination could be restored by the proposed remyelination treatments remains an unanswered question.

Mechanisms of remyelination

Demyelination (induced experimentally or by disease), can be followed by this regenerative response leading to the formation of new myelin sheaths around denuded axons by newly formed oligodendrocytes.[28,208-211] Histopathological assessments have highlighted that this can occur extensively in some people with

MS,[212] but it is inadequate in a significant proportion.[213,214] For example, one study analysed forebrain tissue from 51 MS patients and found widespread remyelination in 20% of individuals, yet 34 cases remyelinated fewer than 25% of their lesions.[214] Such high inter-subject and inter-lesional variability in remyelination capacity is supported by dynamic myelin imaging using positron emission tomography (PET).[215] When combined with evidence that those demonstrating more remyelination display lower levels of disability,[216] it underscores the therapeutic promise of a remyelinating treatment. Consequently, efforts have been made to understand the mechanisms of remyelination and why this fails in MS, in the hope of defining druggable targets to enhance this process.

In animals, pre-existing mature oligodendrocytes are able to increase the number of internodes they generate, and therefore contribute to recovery after demyelination,[217] but they do not add to the pool of new myelinogenic oligodendrocytes that are required for remyelination.[218] Thus remyelination is crucially dependent upon adult oligodendrocyte progenitor cells (aOPCs), themselves derived from neonatal OPCs (nOPCs),[219] which have been shown by genetic fate-mapping to be the cells responsible for generation of the majority of new oligodendrocytes in the adult nervous system.[220,221] These cells are maintained in sufficient quantities predominantly by their own self-renewal, rather than by replacement from neural stem cell niches in the CNS.[222]

Following damage to myelinated areas, aOPCs follow a choreographed process of activation, migration, proliferation and differentiation before culminating in the formation of new myelin sheaths.[220] The final product is a compacted layer of myelin that is thinner and shorter than those formed during developmental myelination,[223] a fact often used to identify remyelination histologically when the process is studied in animal models (Box 1.1). Mechanistically, remyelination might fail due to a defect anywhere in this sequence; a paucity of pro-regenerative factors, or excess of inhibitory factors, as can be seen in MS lesions, combined with the intrinsic composition of the aOPC, can all limit capacity to remyelinate.[28]

BOX 1.1: Animal models used to study remyelination

Remyelination has been studied in several animal models:

- Experimental autoimmune encephalomyelitis (EAE): this model of autoimmune inflammation, driven by injection of a myelin peptide alongside an adjuvant, sees inflammation and remyelination occurring concurrently. However, when used experimentally to explore potential medicines, it is often hard to distinguish an effect of attenuation of inflammation from promotion of remyelination. Hence non-inflammatory models have been developed, as below.
- Gliotoxin injections: lysolecithin and ethidium bromide (EB) are toxic to oligodendrocytes yet spare axons. Experimentally they can be injected into the CNS of animals to induce demyelination. Their particular benefit has been that the kinetics of demyelination and remyelination can be closely studied.[224] The limitation is that the lesions do not necessarily model the complexity of those in multiple sclerosis, which contain a myriad of remyelination inhibitors and inflammatory cells.
- Oral cuprizone administration: dietary ingestion of the copper chelator cuprizone results in demyelination of white matter tracts, particularly in the corpus callosum.[225] It models remyelination, on-going in the face of continued demyelination. However, the normally small diameter axons seen in the corpus callosum makes distinguishing a remyelinated from an unaffected axon challenging, and interpretation correspondingly difficult.

The best model for progressive MS is debated and variations of these employed (reviewed in [226]). For reasons that will become apparent, when studying the underlying mechanisms of remyelination failure in progressive MS, these experiments are best performed in aged animals.

Given that large numbers of aOPCs are seen in chronically demyelinated MS lesions,[227] it is often stated that remyelination fails as aOPCs become quiescent and unable to differentiate. As a consequence, increasing research has been deployed to elucidate the key regulators of differentiation[228-231] and identify agents capable of enhancing this process for clinical use, which is discussed further below.

However, it should be acknowledged that in humans aOPC differentiation may not universally be the rate-limiting step. It has previously been established that aOPCs do migrate to sites of injury and evenly distribute themselves to facilitate remyelination,[232] though they probably do so over short distances.[233] Additionally, while aOPCs are responsible for the majority of remyelination in the adult CNS in experimental models, they may not be the only cell contributing to remyelination in humans. Subventricular zone (SVZ) progenitors are also able to differentiate into myelinating oligodendrocytes within the adult CNS[234] though, while myelin derived

from these progenitors is thicker and more functional than that derived from parenchymatous OPCs,[235] their effects are restricted to areas neighbouring the SVZ.[236] Moreover, two recent papers have provided evidence that mature oligodendrocytes may contribute to lesion repair in humans after all. Yeung and colleagues, utilising a novel method of dating of oligodendrocytes in post-mortem MS brains by analysis of nuclear bomb test derived ^{14}C, indicated that the oligodendrocytes in shadow plaques (areas believed to have undergone at least partial remyelination) were not the result of new oligodendrogenesis.[237] Similarly another study, using single nucleus RNA sequencing in post-mortem MS tissue, showed that oligodendrocytes expressing mature markers could participate in remyelination.[238] This latter study also highlighted changes in oligodendrocyte gene expression profiles between areas of normal appearing white matter of MS brains and healthy controls, implying that the pathology seen in lesions may not reflect the global cellular changes occurring in MS. Therefore, in the adult CNS, it seems probable that both adult OPCs and mature oligodendrocytes, with a small contribution from subventricular zone progenitors, participate in the repair process.

In line with this recent challenge to the view of OPC differentiation as the rate-limiting step in humans, Kuhlmann and colleagues have additionally shown that the barrier to successful remyelination can vary with lesion stage. In an analysis of 153 lesions across specimens from 62 humans, they demonstrated that in active/demyelinating lesions, the process of myelin sheath formation, but not reduced oligodendroglial differentiation, was responsible for the lack of remyelination; oligodendroglia were plentiful and there was minimal OPC differentiation in remyelinating active lesions.[239]

Finally, interactions between other glial cells and OPCs are also being increasingly clearly defined. Reactive astrocytes found at the site of demyelination, for example, secrete inhibitors of remyelination such as Endothelin-1[240] and the recent description of A1 reactive astrocytes, which contribute to the death of oligodendrocytes,[241] needs to be incorporated into the current model of remyelination and potential therapeutic targets explored. In parallel, the demonstration that protein synthesis in OPCs is modulated by axonal action potentials[242] speaks to an underlying symbiosis

between the neuron and the cells responsible for its myelination. In the peripheral nervous system, there is a necessary relationship between axon and Schwann cell, exemplified by dependency on the neuron-derived growth factor Neuregulin 1 to drive peripheral nerve myelination.[243] In the CNS, OPCs are able to differentiate even in the absence of axons[244,245] and, as will be discussed below, in culture are able to myelinate inert axon-like substrates.[246,247] Yet, while oligodendrocytes have a default ability to differentiate and myelinate axons, this is modulated by axon diameter and activity, implying a requirement for intact axons in vivo.[248]

The corollary of these points is that a treatment strategy that enhances aOPC differentiation alone may not be sufficient to address remyelination across a population of heterogenous MS patients with lesions of different ages. It seems increasingly probable that combinations of drugs, acting on different processes, will be required to facilitate remyelination, and that these will be most effective when there is a sufficiently preserved demyelinated axon. This latter point forms the rationale for many phase II studies first focussing on people with RRMS, in whom it is anticipated that fewer axons will have degenerated.

The failure of remyelination in MS

To understand why remyelination fails in MS, one must look at two crucial contributory processes – namely those of age and the immune system.

While the immune system is often seen as having a detrimental role in MS, the innate immune system has been shown to be essential in the biology of remyelination.[249] Myelin debris contains inhibitors of aOPC differentiation and so its clearance, by phagocytosis, is an important step in the regeneration of the myelin sheath.[250-253] Similarly, infiltrating macrophages and activated microglia secrete a myriad of neurotrophic factors, which have direct effects on aOPCs.[254] Indeed, in order to facilitate robust remyelination *in vitro*, the polarization of the macrophage response to an immunoregulatory, “M2”, phenotype is required.[255] It is not clear how these findings relate to the behaviour of monocyte-derived macrophages and

microglia *in vivo*, yet they emphasise how improving our understanding of subpopulations of macrophages/microglia and lymphocytes in the brain is essential to developing treatments that prevent demyelination while promoting remyelination.

The potential for endogenous remyelination is both age- and disease duration-dependent: remyelination is greatest in people aged less than 55 years and within the first 10 years of disease onset.[98,106,210] Disentangling the relative contribution of age versus duration of lesion demyelination to remyelination failure remains to be done, but clinical evidence would suggest age is especially pertinent as patients reach disability milestones at similar ages whether they have relapsing or progressive symptoms at onset.[98,256] Similarly, lesional magnetization transfer ratio (MTR) – a putative marker of remyelination – also shows age-dependent decline.[257] Remyelination is therefore akin to other regenerative processes[258] in becoming less efficient with time;[259-263] I therefore set out to investigate the relationship between age and the treatment response to bexarotene – as measured by MTR and visual evoked potentials – during this PhD (Chapter 3).

Understanding age-associated remyelination failure is essential in treatment development. Mechanistically, in this circumstance, the rate-limiting step is more clearly differentiation of the aOPC, as increasing aOPC recruitment does not lead to enhanced remyelination in aged mice.[264] Studies of how extrinsic factors vary with age have implicated a declining efficiency of the inflammatory response;[265] as noted above, macrophages produce pro-differentiation factors and clear debris by phagocytosis,[253,266,267] which is essential for remyelination. That this process might be modifiable was demonstrated by the reversal of a deficit in remyelination of an aged mouse by twinning its circulation with a young animal by heterochronic parabiosis.[268]

In a similar way, small molecule treatments can be used to promote endogenous remyelination, even in aged animals. A detailed understanding of the intrinsic age-related changes in aOPCs is a recent development in the field following work by Neumann and colleagues.[100] They demonstrated that aged aOPCs become less

responsive to factors that induce differentiation, contributing to the reduced remyelination capacity seen in many non-remyelinating chronic MS lesions.[269] Moreover, RNA sequencing from young and aged aOPCs highlighted a significant contribution from the mTOR pathway. This led to the novel observation that manipulating this pathway in aged rats with caloric restriction (three non-consecutive days of fasting per week over six months), or with the AMPK-agonist metformin (over three months), reverses the diminished differentiation capacity of aOPCs and restores their ability to remyelinate. As a result, manipulation of intrinsic changes in these stem cells is emerging as a promising treatment strategy; one which I intend to test in the development of CCMR Two (Chapter 5).

Finally, there are also anatomical variations to the extent of remyelination within different lesions in the same individual. For example, periventricular lesions are less amenable to remyelination than subcortical lesions,[98,214] while grey matter lesions remyelinate more than those in the white matter.[270,271] This might reflect an underlying heterogeneity in OPCs or in locational differences in permissibility for their differentiation;[272] there are fewer inhibitors of remyelination in the cortex.[270] Or, it could also be due to the importance of neuronal activity to remyelination, which is more likely to occur closer to the soma. Regional variations in remyelination within an individual is an opportunity to investigate barriers to enhancing remyelination, but also raises questions about which lesions should be tested in clinical trials.

Identification of remyelination drugs

An enhanced understanding of the intrinsic and extrinsic regulatory pathways implicated in remyelination has identified a multitude of sensible targets for therapeutic manipulation. An example of this has been the development of opicinumab to inhibit Lingo-1 (leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1), a negative regulator of differentiation.[273]

Another fruitful technique has been high-throughput screening of libraries of compounds, looking for an effect on aOPC differentiation.[274] One such study focussed on the ability of candidate compounds to promote differentiation of rat optic nerve-derived progenitor cells as evidenced by their production of oligodendrocyte differentiation markers.[275] This revealed that antagonism of muscarinic receptors, with the antihistamine/anticholinergic benzatropine, promotes OPC differentiation *in vitro*, which translated into a remyelinating effect in both EAE and cuprizone mice models. Similarly, Najm et al. screened a library of bioactive small molecules, this time on mouse pluripotent epiblast stem cell-derived OPCs.[276] They discovered that the topical corticosteroid, clobetasol, and the anti-fungal, miconazole, as well as benzatropine, leads to a mature oligodendrocyte morphology, and improved remyelination in a lysolecithin-induced mouse model of focal demyelination.

A slightly different approach has used concentric wrapping of myelin around micropillars as an end point rather than differentiation per se. Mei et al. assessed the ability of 1000 FDA-approved small molecules to promote OPCs and oligodendrocytes to ensheath these cone-like structures with myelin.[246] In this way they identified a cluster of compounds with an anti-muscarinic effect: atropine, ipratropium, oxybutynin, trospium, quetiapine, benzatropine and clemastine. This work was quickly translated into the phase II trial of clemastine as a remyelinating therapy,[277] discussed below.

Such small molecules may not have their remyelinating effect through an exclusive action at their canonical targets. The closest to a unifying mechanism has been through demonstration that a wide range of these, including clemastine, benztropine, miconazole and ketoconazole, might promote remyelination through a altering the sterol landscape in the OPC to favour accumulation of 8,9-unsaturated sterols.[278]

However, these techniques predominantly rely on the assumption that OPC differentiation is the rate-limiting step in remyelination[237,238,279]. The micropillar array is also limited in its ability to test the development of functional architecture in the form of nodes and internodes. It follows that combination therapies may be

necessary to optimise an effect across the population of MS lesions. Moreover, such efforts will inevitably be hampered by the lack of an animal model that encapsulates the entire complexity of the MS lesion; there is a risk that agents showing promise in preclinical work do not translate into a beneficial effect in humans or indeed that a potentially useful treatment effect is missed in such models, halting progression towards clinical studies.[28]

Clinical trials of remyelinating drugs

The identification of agents that therapeutically enhance endogenous remyelination in preclinical models has led several to be translated into clinical trials and the possibility of a remyelinating treatment in MS looks increasingly likely. In Table 1.6 I summarise the clinical trials that have been performed while considering a few in more detail below.

Clemastine

This is a first generation anti-histamine that was identified in the micropillar array as being capable of stimulating OPCs to differentiate and carry out the first stages of myelination.[246] This was confirmed in a further screen[275] and shown to occur via an off-target anti-muscarinic action, likely a specific effect on the M1 muscarinic receptor.[34] Ensuing work would confirm its remyelinating effect in multiple animal models.[34,280,281]

As clemastine has been licenced for allergic rhinitis since 1992, it was readily translated into a clinical trial.[277] The ReBUILD study was a single-centre, double-blind, randomized, placebo-controlled, phase II, crossover trial, which specifically investigated the remyelinating potential of clemastine in patients with RRMS and evidence of chronic demyelinating optic neuropathy. Their inclusion criteria ensured that there was detectable demyelination in the optic pathway (evidenced by a visual evoked potential (VEP) P100 latency >118 ms in at least one eye), but also sufficient axons to regenerate (with a retinal nerve fibre layer thickness (RNFL) >70 μm in the qualifying eye when measured with optical coherence tomography (OCT)). Meanwhile, the remyelination that might be expected in the natural history of optic neuritis was excluded by selecting only those without a history of acute optic neuritis in the qualifying eye within the last 5 years, or in either eye in the last 6 months.

The study design saw participants divided into two groups but ensured that all had access to the study drug (which is readily available in the US without prescription). In a double-blind design, 25 were given 5.36 mg of clemastine twice daily for 90 days

followed by placebo for 60 days (group 1), while a further 25 patients were given placebo for 90 days followed by clemastine for 60 days (group 2).

The results of this were rather promising. The primary endpoint, VEP P100 full-field latency, was reduced by 1.7 ms/eye (p=0.0048) in the cross over model. Furthermore, the effect of clemastine on VEP latency was sustained in group 1 after switching to placebo. Thus, the cross-over model underestimates the actual effect, later demonstrated to be a 3.2 ms reduction in P100 latency. Further, there was a significant improvement in a functional outcome, low contrast letter acuity, when the delayed treatment analysis was employed. All the while, the drug was well tolerated, though was associated with fatigue. Secondary endpoints were negative however, including MRI assessments of myelin water fraction (MWF), whole brain MTR, white matter MTR, and white matter fractional anisotropy (FA). There was no effect on the expanded disability status scale (EDSS), a timed 25-foot walk or the 6-minute walk test.

This positive trial has provided some optimism about clemastine, though its selective inclusion criteria, investigating just those persons with chronic optic neuropathy, raises the possibility that the results are not generalisable; 75 patients were excluded because their VEPs did not meet the threshold latency of 118 ms. The ReCOVER trial (NCT02521311) is currently testing clemastine in patients following a relapse (4mg three times daily for 1 week, followed by 4mg twice daily until 3 months following an episode of acute optic neuritis). While promising, there is still much work to be done: clemastine requires progression to phase III studies, it should be trialled in progressive cohorts, and the possibility of combining treatments, such as with the potential synergistic effect of metformin, requires investigation (Chapter 5).

Opicinimab

As mentioned above, Lingo-1 is a negative regulator of oligodendrocyte differentiation and its antagonism has been shown *in vitro* and in animal models of CNS demyelination to enhance remyelination.[282] The human monoclonal antibody opicinumab (anti-Lingo-1) showed remyelinating activity in preclinical studies[283] and

therefore its utility was explored in early clinical trials. After passing safety analyses in a phase I trial,[284] there was a phase II, randomized, double-blind, placebo-controlled, clinical trial (RENEW) in 82 patients with a first episode of acute optic neuritis (but not necessarily a diagnosis of MS).[285] The primary outcome measure was the recovery in VEP P100 latency in the affected eye, referenced to the unaffected eye, over 24 weeks of treatment (at 100 mg/kg) after an episode of optic neuritis. The mean treatment difference of opicinumab (17.3 ms) versus placebo (20.8 ms) was -3.5 ms (95% CI -10.6, 3.7; p=0.33) in the ITT population, and –7·6 ms in the PP population (95% CI -15.1 to 0.0; p=0.050). The authors attributed these between-sample differences to adverse events leading to early withdrawal at a time when latency delay was severe: this applied to four (10%) participants in the placebo group and seven (17%) participants in the opicinumab group. No change was observed in the secondary endpoints, though this did not include MRI sequences such as MTR.

The SYNERGY trial followed: a dose-ranging study including 418 people with RRMS and SPMS whom were taking interferon-β1a.[286] The primary outcome measure was the percentage of participants achieving confirmed disability improvement over 72 weeks, measured as a composite of ambulation (25-foot walk), upper extremity function (9HPT), cognition (3-Second Paced Auditory Serial Addition Test; PASAT) and the EDSS. Confirmed disability improvement was seen in 49% of participants on placebo, 47% on opicinumab 3 mg/kg, 63% on 10 mg/kg, 65% on 30 mg/kg, and 40% on 100 mg/kg; a linear dose response was not seen but the increase in percentage of responders in those treated with the mid-range doses of 10 and 30 mg/kg has led to Biogen proceeding with a refined phase II trial (AFFINITY) in addition to an extension study (RENEWED, NCT02657915) of the RENEW trial.

GSK239512

This H3-receptor antagonist was originally developed to treat Alzheimer's disease[287,288] and was put forward as a potential remyelinating agent because H3 negatively regulates oligodendrocyte differentiation.[289] A phase II, randomised, placebo-controlled study in people with RRMS on interferon or glatiramer acetate,

tested the remyelinating effects of one-year of treatment with GSK239512 on acute lesions measured through two co-primary endpoints. First, the mean change in MTR post-lesion compared with pre-lesion in newly developed (on trial) gadolinium-enhancing (GdE) lesions. And second, the mean change in MTR for acute lesions defined by Delta-MTR: regions experiencing a decrease greater than the 99th percentile of the normal variation measured in white matter from one scan to the next.[257] Among the secondary outcomes was the mean change in (chronic) T2 lesion MTR. The mean change in MTR was modelled separately for each lesion within each patient, allowing for variation in effects between patients and between different lesions, within patients. Treatment effects, relative to placebo, were given as the treatment difference divided by the standard deviation (estimated as the between-group variability) of the treatment difference. From 131 randomised participants, 92 GdE lesions from 27 patients and 69 Delta-MTR-defined lesions from 24 patients were identified in the GSK239512 group. Meanwhile, 97 GdE lesions from 28 patients and 77 Delta-MTR lesions from 29 patients in the placebo group were included. The active arm was associated with positive effect sizes of 0.344 (90% CI 0.012, 0.671) and 0.243 (90% CI, -0.112, 0.598) for GdE and Delta-MTR lesions respectively.[290] However, there was no active-placebo difference in mean MTR change for chronic T2-weighted lesions: -0.022 (90% CI -0.052, 0.009) percentage units. While this trial indicated a statistically significant effect of the drug on GdE lesions, fewer than half of the participants contributed to the analysis, raising questions about generalisability of the results. The trial design additionally highlighted the low yield of acute lesions at MRI in participants on (even low-efficacy) DMTs; as discussed below 1179 MRI brain scans were undertaken in this trial to detect just 189 acute lesions.

Bexarotene

Another positive regulator of OPC differentiation is the retinoid acid receptor (RXR)-γ.[291] In remyelinated MS lesions, RXR-γ is expressed in cells of the oligodendrocyte lineage, and knockdown of the receptor in culture inhibits human OPC differentiation. Meanwhile, administration of the RXR agonist 9-cis-retinoic acid to demyelinated cerebellar slice cultures, and to aged rats after demyelination, promotes

remyelination.[291] There are no licensed selective RXR-γ agonists[292] but bexarotene, a non-selective agonist of the α, β, and γ retinoid X receptors (RXR), is licenced to treat cutaneous T-cell lymphoma.[293] The results of the CCMR One study, which tested the effects of bexarotene on demyelinated lesions, are presented in Chapter 2 of this thesis.

Biotin

Biotin is postulated to promote remyelination when given in high-doses through its role as a cofactor for carboxylases required for fatty acid synthesis in oligodendrocytes.[294] To date, clinical trials have mostly focussed on cohorts of progressive patients. In the MS-SPI study, 13 (12.6%) participants in the active arm (compared to 0 in the placebo arm) achieved the primary endpoint of sustained disability reversal (decrease in EDSS or timed 25-foot walk).[295] Unfortunately, this failed to be replicated in the ensuing MS-SPI2 study.[296] The MS-ON study similarly returned a negative result when change in visual acuity was employed as the primary endpoint.[297] It therefore seems unlikely that high-dose biotin could be a clinically useful treatment for people with MS.[298]

Cell-based therapies

Outside of enhancing the activity of endogenous oligodendrocyte progenitors, other non-ablative cell-based approaches are generating substantial interest (reviewed in [299]): transplantation of mesenchymal stem cells (MSCs), derived from bone marrow or other tissues, or transplantation of OPCs, derived from foetal tissue, embryonic stem cells or induced pluripotent stem cells (iPSCs)[300,301] are viable options, but remain experimental. Challenges exist for each with regards to cell production, mode of delivery, tumour-forming potential, and requirements for immune suppression, although using autologous sources may abrogate the need for the latter. One noteworthy, albeit uncontrolled, trial administered bone marrow-derived MSCs to ten patients with progressive MS, noting an improvement in VEP latency of 1.3 ms, interpreting the mechanism for this as a neuroprotective effect through the promotion of myelin repair.[302] Larger phase II studies are underway,[303] though there are many unresolved barriers to widespread application of a transplant-based approach MS.

Treatment (trial name, NCT ID)	Information	Primary outcome	Status/result (references)
Liothyronine (MST3K, NCT02760056)	A phase I, randomised, double-blind, placebo-controlled, dose-finding trial of liothyronine sodium (L-T3) given for one week to people with any type of MS	Maximum tolerated dose (MTD) of L-T3 as measured by hyperthyroid symptom scale	Completed. MTD was 75mcg once daily. No significant difference for LCLA and VEP latency[304]
CNM-Au8 Nanocrystalline gold (NCT03536559)	A phase II, randomised, double-blind, placebo-controlled, parallel group study in 150 people with MS and evidence of chronic optic neuropathy.	Change in LCLA. Secondary outcome: change in multifocal visual evoked potential latency at 24 weeks	Recruiting
Bexarotene (CCMR One)	A phase II, randomised, double-blind, placebo-controlled trial of 50 people with RRMS treated with dimethyl fumarate	Change in mean lesional MTR in chronic lesions with an MTR below the within-patient median	Completed. Results in this thesis
Adrenocorticotrophin, ACTH (NCT02446886)	A phase IV, randomised, open-label study of ACTH gel on remyelination in patients with RRMS or SPMS and new contrast-enhancing lesions	Change in MWF within new Gd-enhancing lesions over the course of 12 months	Completed June 2020. Results awaited.
GSK239512 (NCT01772199)	A phase II, randomised, placebo-controlled, single-blind study in 131 people with RRMS on interferon or glatiramer acetate	Mean change in Gd-enhancing lesion MTR from before enhancement to stable recovery.	Completed. Positive effect observed on Gd-enhancing lesions[290]

Biotin/MD1003 (MS-SPI, NCT02220933)	A phase III, randomised, placebo-controlled, double-blind trial of high-dose biotin in 154 people with SPMS or PPMS	Disability reversal with EDSS decrease of >1 or >20% increase in T25FW	Completed. 12.6% of treated patients achieved primary endpoint versus none of the untreated patients[295]
Biotin/MD1003 (MS-SPI2, NCT02936037)	A phase III, randomised, placebo-controlled, double-blind trial of high-dose biotin in 642 people with SPMS or PPMS	As above	Completed. No difference between active and placebo arms[296]
Biotin/MD1003 (MS-ON, NCT02220244)	A phase III, randomised, placebo-controlled, double-blind trial of high-dose biotin in 93 people with SPMS or PPMS	Change in visual acuity over 6 months	Completed. No significant changes in visual acuity[297]
Olesoxime (MSREPAIR, NCT01808885)	A phase Ib, randomised, placebo-controlled, double-blind trial of olesoxime compared to placebo in 44 people with RRMS	Safety criteria, though MTR included in exploratory outcome measures	Completed. Safe and well tolerated. No between group effects seen[305]
Clemastine (ReBUILD, NCT02040298)	A phase II, randomised, placebo-controlled, double-blind, crossover trial in 50 people with RRMS and chronic stable optic neuropathy.	Change in P100 latency of the full-field VEP	Completed. Significant latency reduction of 1.7 ms in the crossover model and 3.2 ms in delayed treatment analysis[277]
Clemastine (ReCOVER, NCT02521311)	A phase II, randomised, double-blind, placebo-controlled trial in 90 people diagnosed with acute demyelinating optic neuritis	Change in P100 latency of the full-field VEP and change in low contrast visual acuity	Recruiting

Quetiapine (NCT02087631)	A phase I/II open label, dose-ranging study of quetiapine in people with RRMS and PMS	Dose-limiting toxicity, no specific remyelination outcomes	Completed. Drug not tolerable at low doses[306]
Opicinumab (RENEW, NCT01721161)	A phase II, randomised, placebo-controlled, double-blind, study of opicinumab in subjects with a first episode of acute optic neuritis	Change in VEP P100 latency in affected eye, referenced to the unaffected eye, over 24 weeks of treatment	Completed. Significant improvement in latency, but only on per protocol analysis[285]
Opicinumab (SYNERGY, NCT01864148)	A phase II, randomised, placebo-controlled, double-blind trial of opicinumab (at 3, 10, 30, or 100 mg/kg) in 418 subjects with RRMS treated with interferon β1a	Change in performance at EDSS, T25FW, 9HPT and 3s-PASAT	Completed. Did not meet primary endpoint, but increased percentage of improvement responders at 10 and 30 mg/kg doses[286]
Opicinumab (AFFINITY, NCT03222973)	A phase II randomised, double-blind, placebo-controlled study of opicinumab versus placebo in 263 people with RRMS	Overall response score composed of EDSS, T25FW and 9HPT from each hand	Ongoing
rHIgM22[307] (NCT02398461)	A phase I randomised, double-blind, placebo-controlled study of rHIgM22 compared to placebo in 72 people with RRMS following a relapse	Safety and tolerability endpoints	Completed. No significant safety signals[308]

Domperidone (NCT02493049)	A phase II randomised, open-label, single-blind study of domperidone 10mg three times daily in people with RRMS and new Gd-enhancing lesions	Texture analysis, DTI and MTR in enhancing lesions over 32 weeks	Completed. Results awaited.
Transorbital electrical stimulation (ONSTIM, NCT04042363)	A randomized, controlled, blinded trial of 10 sessions of transorbital electrical nerve stimulation over 2 weeks after an acute episode of retrobulbar optic neuritis	Change in VEP P100 latency	Recruiting
Bazedoxifene Acetate (ReWRAP, NCT04002934)	A randomized, placebo controlled, double-blind, trial of BZA in 50 women with MS	Change in VEP P100 latency	Recruiting

Table 1.6. Clinical trials of remyelination treatments in multiple sclerosis. MTD, maximally tolerated dose; NABT, normal appearing brain tissue; MTR, magnetization transfer ratio; LCLA, low contrast letter acuity; Gd, gadolinium; MWF, myelin water fraction; DTI, diffusion tensor imaging; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PMS, progressive multiple sclerosis; IFN, interferon; VEP, visual evoked potential; EDSS, expanded disability status scale; T25FW, timed 25-foot walk; 9HPT, 9 hole peg test; PASAT, paced auditory serial addition task.

Outcome measures for remyelination trials

One of the foremost challenges to translating promising preclinical findings into clinical studies is uncertainty in the optimum way to demonstrate a remyelinating effect in living individuals. Given that the anticipated benefit to the patient – the prevention or delay to progression – only manifests over years, reliance on standard objective clinical markers of disability, for example the EDSS,[309] as outcomes may miss a useful therapeutic effect over a comparatively short clinical trial. While such disability measures are undoubtedly of more importance to the patient, they are more suited to later stages of drug development; as established in the previous section, we are still at the stage of identifying agents which have any biological effect on remyelination. Using functional scores to study remyelination specifically is further complicated by other adaptations that occur in nerves in response to injury, such as ion channel redistribution and cortical plasticity/adaptation after demyelination.[310,311]

There follows a reliance on paraclinical measures to determine a treatment effect. With no accessible tissue for histological examination, a combination of neurophysiological and imaging-based assessments that are more direct assessments of the pathological processes being targeted are typically required.[312] Of course, a related barrier to successful translation is uncertainty in the amount of remyelination that would be clinically meaningful, a point I will return to in my conclusions. Here, I describe the most prevalent outcome measures being used in phase II trials.

Neuroimaging

From the imaging perspective, standard MRI sequences can be deployed to measure the number and size of lesions in the white matter (WM) and grey matter (GM), as well as atrophy of the brain and spinal cord. Yet these measures (such as T2 weighted and gadolinium-enhanced T1 weighted sequences) correlate only modestly with disability and lack the ability to differentiate between the different pathological correlates of MS: namely inflammation, oedema, axonal loss,

demyelination, remyelination and gliosis.[313] As a result, advanced MRI techniques that interrogate tissue microstructure – including myelin water fraction (MWF),[314,315] diffusion tensor imaging (DTI)[316,317] and magnetization transfer ratio (MTR),[318,319] as well as positron emission tomography (PET),[215,320] have been used to measure myelin dynamics.

Lesions on MRI

WM lesions are the most readily identifiable MRI abnormality in MS, though resolution at 1.5T still fails to identify a significant proportion: T2-weighted sequences can identify 63% of histopathologically demonstrated WM lesions, while 3D FLAIR increases this to 71%.[321] Identifying GM lesions is more challenging still. For mixed GM-WM and deep GM lesions pathological studies suggest this figure is in the region of 40%. Yet fewer than 5% of cortical lesions can be identified with T2 and 3D FLAIR.[321] Additional sequences, such as double inversion recovery (DIR) and phase sensitive inversion recovery (PSIR), can improve sensitivity to detecting cortical GM lesions,[322,323] but even when these techniques are deployed with 3T, no more than 20% of cortical GM lesions are seen.[324] This is further compounded by the finding that fewer than 11% of subpial (type III) GM lesions, which account for two-thirds of all cortical lesions in people with progressive MS,[92,93] are detectable with 3T MRI.[324] Consequently, MRI-determined normal appearing grey matter (NAGM) will contain significant numbers of occult lesions, and this may be more marked in the subpial region.

Another consideration when selecting lesions that might be most amenable to remyelination is establishing their chronicity. The administration of intravenous gadolinium-based MRI contrast can lead to enhancement of new WM lesions for 2-8 weeks, although typically <4 weeks.[325] Additional methods of identifying acute lesions involve serial imaging, which enables detection of new areas of T2-hyperintensity, or isolation of regions that experience a decrease in MTR (for example, a decrease greater than the 99th percentile of the normal variation measured in WM from one scan to the next).[290] Unfortunately, the limitation of an imaging approach to a remyelination trial of acute lesions, is the necessity for

frequent MRI scans: Schwartzbach et. al. required their 131 participants (on low-efficacy DMTs) to undergo 9 MRI scans every 6 weeks in their clinical trial of GSK239512, in order to detect 189 acute lesions.[290] This return would be likely be even lower if participants were on more effective DMTs.

Magnetization transfer ratio

Magnetization transfer techniques investigate the exchange of magnetisation between protons in at least two pools: those that are mobile and those associated with macromolecules such as myelin or axonal membranes. Expressed as a ratio (MTR), it provides a quantitative measure of the proportion of protons bound to macro-molecular structures relative to those that are free in water and has been demonstrated to correlate with pathological quantification of myelin in both WM and GM lesions: demyelinated lesions have a significantly lower MTR than remyelinated lesions.[319,326,327] MTR can be used to quantify myelin in several ways. One can use serial measures of mean MTR in white and grey matter,[328] in chronic lesions,[329] or in acute (gadolinium-enhancing) lesions.[257] Indeed, as not all lesions remyelinate to the same extent, further refinements have been proposed. For example, in the CCMR-one study of bexarotene (Chapter 2), the primary outcome measure was based on the change in mean MTR of established lesions with a low MTR at the baseline scan, thereby maximising sensitivity to an effect on lesions that are demyelinated at the outset.[329] These aforementioned techniques have been used to show that mean MTR in white and grey matter remains static over time in people with MS treated with alemtuzumab, but deteriorates if not on disease modifying treatment,[328] and that the anti-histamine GSK239512 has a positive effect on mean MTR in gadolinium-enhancing lesions.[290]

Diffusion tensor imaging

Alternatively, DTI provides information about tissue microstructure by measuring water diffusion *in vivo.*[330] Parameters derived from this include radial diffusivity (a marker of water motion perpendicular to the axon), mean diffusivity (an index of water diffusion regardless of direction), axial diffusivity (a marker of water motion parallel to the axon) and fractional anisotropy (a measure of the directionality of

water movement). Combined MRI and histopathological studies show that fractional anisotropy (FA) correlates with both axonal counts and myelin content,[331,332] while radial diffusivity increases in demyelinated areas in experimental models.[317] However, a limitation of this technique is that these measures depend on the ability to detect the correct fibre-tract orientation per voxel, otherwise complex tissue microstructure such as crossing fibres, rather than pathology specifically, can impact on diffusivity.[333] As a result, DTI is perhaps best used to assess white matter tracts such as the visual pathway;[334] an attractive prospect given the potential to deploy this alongside functional measures of the same tract using visual evoked potentials.

Myelin water fraction

The myelin water fraction (MWF) infers the proportion of water trapped between myelin bilayers ('myelin water') relative to water inside and outside of axons (which have longer T2 relaxation times), and can be also as a proxy for myelin content.[314,335] The multi-component T2 relaxation technique required for this is, however, constrained by long acquisition times, and there remains no histopathological evidence of greater sensitivity or specificity to myelin than MTR.

Positron-emission tomography (PET)

Outside of MRI, PET imaging, alongside a myelin-specific ligand such as Pittsburgh compound B ([11C]PiB), has the capacity to measure the extent of demyelination without incorporating biases from other pathological changes, such as gliosis or axonal loss.[336] PiB binds in decreasing amounts to MRI-determined NAWM, gadolinium-enhancing lesions, perilesional WM, T2 lesions, and T1 black holes,[215] a pattern that has been replicated with other ligands.[337] In addition to affording the potential to explore changes over repeated scans, PET could also potentially be used to stratify the patients by their remyelination potential for clinical studies.[215] However, the issues of ligand (which often requires on site synthesis due to short half-lives) and scanner availability, as well as the consequences of radiation exposure, are likely to limit the role of PET to research studies.[338]

Brain atrophy

Finally, brain atrophy, though clearly not a direct measure of remyelination, reflects the neuronal and axonal losses that a remyelinating therapy ultimately endeavours to prevent.[339] Evident from the earliest stages of MS,[340] brain atrophy continues at a rate of around 0.5-1% each year in people with MS,[341] compared to around 0.1-0.3% per year in healthy controls,[342] and occurs more in the GM than WM.[343] It is typically assessed using semi-automated methods applied to the T1-weighted sequences, such as the SIENA[344] and, for a 2-year trial duration, can be used as an outcome measure with feasible sample sizes.[345] While unlikely to demonstrate an effect within a short phase II remyelination trial, brain atrophy is a sufficiently robust and clinically relevant outcome measure to be deployed as the primary outcome for both phase II and III trials – as it was in the MS SMART clinical trial.[119,346]

The eyes and visual function

Visual evoked potentials

Evoked potentials allow for an assessment of nervous conduction along visual, somatosensory, auditory, and motor tracts in a way that correlates with function[347-349] and disability,[350] but their clinical utility, particularly in diagnostics, has largely been replaced by MRI.[351] However, such indices are proving invaluable as biomarkers in assessing remyelination; in the recent positive phase II trial of clemastine, it was a reduction in VEP P100 latency, rather than clinical or imaging markers, that confirmed the biological effect.[277,352]

The pattern reversal full-field VEP represents the averaged recordable electric potential in the visual cortex in response to the presentation of an alternating checkerboard-patterned stimulus. VEPs are understood to be generated at the level of the striate cortex by the combined activity of postsynaptic potentials.[353] The latency of the VEP consequently reflects the speed of conduction among the fastest conducting fibres along the retino-geniculate-striate pathway, while the magnitude of the VEP reflects the number of functional afferent fibres reaching the striate cortex and the degree of synaptic activity in V1. This technique is therefore a sensitive and

objective tool for quantifying dynamic changes in myelination through latency abnormalities in the visual pathway, while also being able to monitor conduction block and axonal loss through changes in amplitude.[354]

In remyelination clinical trials, the main focus is on the positive deflection in the VEP waveform approximately 100 ms after the visual stimulus (the P100), which is the most reproducible part of the response.[355] Following an attack of optic neuritis, VEP latencies are prolonged but a period of recovery follows, most significantly within the first 6 months, but for perhaps as long as 2 years.[356,357] In contrast, in those with chronic stable optic neuropathy, a prolonged P100 latency is seen, which has been shown in longitudinal data to remain stable, or gradually lengthen, with time.[358] As a result, in studies of patients without a recent bout of optic neuritis, improvements in VEP P100 latency can be used as a marker of remyelination; this was the rationale behind the ReBUILD trial.[277] When studies have enrolled patients with acute optic neuritis meanwhile, such as in the RENEW study of opicinumab,[285] values for the unaffected contralateral eye have been used as a control, and the outcome measure given as the change in latency difference between the two eyes.

However, a limitation of full-field VEP is that macular overrepresentation in the visual cortex weights any latency abnormalities significantly to those in the central field; small or localised optic pathway demyelination or axonal loss is often not detected.[359,360] This problem is compounded by the conventional electrode placement (frontal-occipital) which favours the response from the lower visual field.[361] A further limitation, also attributable to the anatomy of the visual pathway, is phase cancellation. The retinotopic projection to the visual cortex means that the upper visual field projects to the lower bank (lingual gyrus) of the sulcus calcarinus, while the lower visual field projects to the upper bank (cuneus gyrus). As these face each other, the cortical dipoles from the upper and lower hemifields are almost opposite, resulting in a cancellation effect of amplitude in the unaffected eye.[353] Accordingly, another consequence of damage to a discrete location in the visual pathway, such as a multiple sclerosis lesion, can be that the recorded signal appears larger due to less cancellation effect.

By contrast, the technique employed in multifocal VEP (MF-VEP) is to simultaneously stimulate multiple individual regions of the visual field.[362] By cross-correlating each sequence with the raw EEG signal, responses to unique sequences corresponding to each of the individually stimulated segments are extracted. This allows for an assessment of a much larger cross sectional area of the optic nerve, with better identification of regional changes in latency and amplitude, and potentially a more precise analysis of injury and repair following optic neuritis.[363-365] For example, MF-VEP was deployed in a substudy of the RENEW trial of acute optic neuritis to compare mean changes in affected and fellow eye latencies and amplitudes from fellow eye baseline latency and amplitude.[366] This showed trends to latency improvement (mean change of -11.78 ms between opicinumab and placebo (95% CI -24.28, 0.73, p=0.06)) and amplitude recovery (mean gain versus placebo was 22.32 nV (95% CI -1.26, 45.89 p=0.06)), but significant variation between subjects led the study authors to conclude that they were underpowered with only 39 participants. It is now being used in clinical trials as a main outcome measure, such as in the clinical trial of nanocrystalline gold (NCT03536559), and will be similarly utilised in the CCMR Two trial of metformin and clemastine (Chapter 5).

Optical coherence tomography

Optical coherence tomography (OCT) is another tool being employed to study neural degeneration and regeneration in MS, and thus indirectly assess remyelinating therapies. This technique acquires high resolution images of the retina, allowing the measurement of the thickness of the retinal nerve fibre layer in both the peripapillary region and the macula (pRNFL and mRNFL; reflecting the integrity of axons), as well as the ganglion cell layer (GCL; neurons) and inner plexiform layer (IPL; dendrites).[352] Myelination in the visual pathway begins at the lamina cribrosa, slightly behind the eye, and thus visualisation of these retinal layers provides insight into the proximal effects of a disease process that is most often found in the retrobulbar portion of the optic nerve.[367]

RNFL thickness is decreased following optic neuritis on account of retrograde axonal degeneration,[368] and thinning occurs in people with MS even in the absence of ON.[369] In a meta-analysis in 2010, Petzold showed that eyes with a history of AON had pRNFL thinning by a mean of 20.38 µm (95% CI 17.91, 22.86 µm), while MS eyes without an ON history had an average thinning of 7.08 µm (95% CI 5.52, 8.65 µm) compared to disease-free controls.[370] For comparison, a normal pRNFL thickness is in the region of 105 µm, though there is variation between individuals and physiological loss due to aging (approximately 10-20 µm loss over 60 years).[371] In terms of the time course of these changes after acute ON, Costello et al. showed that the majority sustain 10-40 µm pRNFL thinning within 3 to 6 months,[372] while Henderson et al. showed the mean time to 90% maximum pRNFL loss was 2.38 months.[373] An additional important finding from the former study was the demonstration that 75-80 µm is something of a threshold level, below which there are severe decrements in visual function;[372] as described above, in the ReBUILD study, a RNFL of >70 µm was required for an eye to be included in their study.[277]

Yet, while the RNFL is a good measure of axonal degeneration, the GCL is perhaps the most attractive layer to monitor ON, since it is not affected by oedema and so its thinning is not masked by early inflammation.[374] It also correlates better with markers of visual function, such as low-contrast letter acuity, in people with MS.[375] This was the rationale for the RENEW study, which recruited people with acute ON to receive opicinumab, to monitor GCL/IPL thickness as a secondary outcome; no treatment effect was seen.

In any case, while OCT outcomes are not themselves intrinsically representative of myelination, they may measure secondary neuroprotective effects. Thus far, no clinical trials of a remyelinating drug have shown a positive effect using OCT, and its use is perhaps best served as a selection criterion to ensure there are sufficient axons to remyelinate.[277]

Visual acuity

Tests of low-contrast vision, in particular low-contrast letter acuity (LCLA), have a greater capacity to detect visual impairment in MS than high-contrast letter acuity (HCLA).[376,377] MS patients have significantly lower LCLA scores than disease-free controls[378,379] and, while visual recovery following AON is often said to be good with HCVA,[380,381] studies in AON have shown persistent visual deficits in LCLA.[382] Sloan LCLA charts were first used as an outcome in the IMPACT study of interferon β-1a, in which they showed superior performance in detecting worse visual function compared to Pelli-Robson contrast-sensitivity charts, and assessments of colour vision (L'Anthony D-15 DS colour test) and visual fields (Esterman binocular test).[383] Other studies have also revealed correlations of Sloan LCLA with OCT, MRI, VEP, and disability (both with the EDSS and MSFC).[384] Consequently, Sloan LCLA has been increasingly used as an outcome measure, and proven sensitive to treatment effects in several clinical trials.[385]

Sloan LCLA charts follow the standardised format of the Early Treatment Diabetic Retinopathy Study (ETDRS)[386] and come in a variety of different contrast thresholds ranging from 1.25% to 100%. They are superior to standard Snellen charts: (i) each optotype is equally detectable for normal observers; (ii) each line has an equal number of letters (five per line); (iii) each line is spaced in equal logarithmic steps of the visual acuity (the resolvable angle). Meanwhile, reliability testing has led to the consensus that a loss of seven letters is a clinically meaningful change.[378]

In remyelination trials, a positive effect on LCLA was seen as a secondary outcome of the ReBUILD study of clemastine: an increase of 1.6 letters per eye (95% CI 0.2, 3.0; p=0.022) was observed.[277] LCLA is also being used as the primary outcome measure for the clinical trial of nanocrystalline gold (Table 1.6; NCT03536559). It therefore remains an important component of the assessment of response to a putative remyelinating therapy.

Other techniques

Multi-modal evoked potentials

The clinical heterogeneity of MS has already been described and extends beyond a consideration of optic neuritis; it is important to know if other tracts might be sensitive to change in remyelination trials. Given the emerging importance of neurophysiological measures in such studies, a potential biomarker in future trials might be a combination of VEPs, motor EPs (MEPs), somatosensory EPs (SSEPs), and brainstem auditory EPs (BAEPs).[387] Such "multimodal" evoked potentials can be combined to give a "global" outcome, which has previously been shown to correlate with disability and inform disease progression,[388] and have already been employed in the field of bone marrow-derived cell therapy.[303,389]

Oculometry

Meanwhile, other exploratory neurophysiological techniques have also been emerging as potential methods for detecting the functional consequences of remyelination. For example, a recent clinical study utilised measurements of saccadic eye movements in people with internuclear ophthalmoparesis to demonstrate improved conduction along the medial longitudinal fasciculus when treated with fampridine.[390] While fampridine's mechanism of action is through potassium channel blockade, the authors advocated the MLF as an additional MS-relevant tract to monitor the consequences of remyelination.

Additionally, it has previously been suggested that serial monitoring of saccadic latency parameters might be a sensitive measure of neuroprotection.[391] One group has published four studies from one cohort of 25 subjects with MS.[392-395]. They reported to deficits in complex decision making, such as prolonged latency and increased proportion of errors (prosaccades) in the antisaccade task.[394] They also reported that latency of memory guided saccades correlated with EDSS.[393] In contrast to evoked potentials, saccadic latency depends on a large network of diffuse pathways. Yet, while an increase in latency over time could be due to multiple

aetiologies, a shortening in latency is likely to be more specifically due to remyelination; this has not been tested to date.

Neurofilament

Finally, it is noteworthy that there is currently no biomarker of myelin regeneration in biological fluids. That said, neurofilament light, a marker of axonal damage that correlates with MS disease activity and disability,[396,397] has been postulated to be a valuable outcome measure for remyelination trials. The advent of high-sensitivity serum assays has made this increasingly simple to monitor axonal injury in people with MS.[398] A subset of participants from the SYNERGY trial,[286] for example, showed a trend toward neurofilament light decline among treatment responders.[399] However, while serum neurofilament light measures the desired outcome from remyelination – the prevention of axonal degeneration – it is presently unvalidated in remyelination trials and, until that point, should remain exploratory.

Research questions

1. Does bexarotene promote remyelination in people with relapsing remitting multiple sclerosis?
2. Is the remyelinating effect of bexarotene affected by age?
3. Is the remyelinating effect of bexarotene, as measured by visual evoked potentials, durable?
4. Does the combination of metformin and clemastine promote remyelination in people with relapsing remitting multiple sclerosis?
5. Which drugs should be repurposed or rescued for testing in clinical trials of people with progressive multiple sclerosis?
6. What were the barriers to recruitment to interventional trials of COVID-19 during the first wave of the pandemic in England; how might these impact the delivery of these studies?
7. How are saccadic latency distributions affected by MS, do they correlate with more established measures of remyelination?

Chapter 2: The Cambridge Centre for Myelin Repair trial number One (CCMR One)

A randomised placebo-controlled trial of a retinoid-X receptor agonist's ability to promote remyelination in people with relapsing-remitting multiple sclerosis

Abstract

The retinoid acid receptor RXR-γ is a positive regulator of oligodendrocyte precursor cell differentiation and remyelination in vitro, in animal models, and in human cells. Here, we assessed the safety and efficacy of bexarotene, a licensed non-selective RXR agonist, as a treatment for people with relapsing remitting multiple sclerosis.

In this double-blind phase IIa trial (CCMR One, ISRCTN14265371) people with relapsing remitting MS aged 18-50 years, who had been on dimethyl fumarate for ≥6 months, were randomly assigned bexarotene 300 mg/m^2 or placebo for 6 months. The primary efficacy outcome was change in mean lesional magnetization transfer ratio (MTR) in submedian lesions (lesions below the baseline within-patient median MTR), analysed by intention to treat.

52 participants were randomised. All those on bexarotene experienced adverse events: secondary (central) hypothyroidism (n=26, 100%), hypertriglyceridaemia (n=24, 92%), rash (n=13, 50%) and neutropenia (n=10, 38%). The primary efficacy outcome was not met: the bexarotene-placebo difference in adjusted mean submedian lesional MTR change was not statistically significant: 0.16 (95% CI -0.39, 0.71) pu, p=0.554. However, prespecified exploratory analyses of all lesions found a statistically significant difference in treatment effects between submedian and supramedian lesions (interaction p=0.007) and a statistically significant MTR treatment effect on lesions in cortical grey matter (1.00 (95% CI 0.17, 1.83) pu,

p=0.023), deep grey matter (1.93 (95% CI 0.28, 3.59) pu, p=0.027) and the brainstem (1.75 (95% CI 0.86, 2.63) pu, p=0.0004); interaction p<0.0001. Bexarotene also reduced mean adjusted full-field visual evoked potential latency in eyes with prolonged baseline latency by 4.06 ms/eye (95% CI -7.68, -0.44; p=0.028).

This is the first trial to show evidence of remyelination with converging evidence from both MRI and electrophysiology and, while poor tolerability of bexarotene will limit its clinical use, it provides compelling motivation for the development of RXR-γ-specific agonists and a framework for assessing remyelinating therapies.

Contribution statement

I was an evaluating physician and sub-investigator on this trial, recruiting and seeing participants, and monitoring drugs, for those based in Cambridge. I oversaw the VEP data acquisition and analysed the electrophysiology trial outcomes. I was additionally responsible for analysing the safety outcomes of the trial. I wrote the first draft of the trial manuscript, compiled all of the edits, submitted this for publication, and led the ensuing revisions. I was not involved in the development or set-up of this trial, which predated my PhD. I was not involved in the analysis of the trial MRI brain scans.

Background

Although many licensed drugs reduce inflammation effectively,[177] they leave persistently demyelinated axons, which slowly degenerate through loss of trophic support, causing progressive worsening of disability.[29] The most effective strategy to preserve demyelinated axons, and so delay or prevent disability progression, is to enhance endogenous remyelination.[400,401] As OPCs are often found in chronically demyelinated MS lesions,[28] it is widely held that remyelination failure can be attributed in part to impaired OPC differentiation.

Studies to identify therapies capable of enhancing this rate-limiting stage[246,275] have led to a number of clinical trials to treat chronic and acute demyelinating injuries,[277,285,286,290] but only one was published prior to commencement of CCMR One: the phase II study of GSK239512, an H3 receptor antagonist, had shown a statistically significant improvement in the magnetisation transfer ratio (MTR) characteristics of gadolinium-enhancing lesions.[290] Evidence emerging since then has included the phase II ReBUILD study of clemastine, which demonstrated a significant improvement in the latency of the full-field visual evoked potential (VEP),[277] and the phase II RENEW trial of opicinumab (anti-Lingo1), which showed an improvement in VEP latency using a per protocol analysis of participants with acute optic neuritis.[285]

Another positive regulator of OPC differentiation is the retinoid X receptor (RXR)-γ,[291] which is expressed in remyelinated MS lesions in oligodendrocyte lineage cells. Inhibition of RXR-γ signalling inhibits differentiation of rodent and human OPCs;[402] and the RXR agonist, 9-cis-retinoic acid, remyelinates both demyelinated cerebellar slice cultures, and focal toxin-induced demyelination in aged rats.[291] There are no licensed selective RXR-γ agonists;[292] however bexarotene, a non-selective agonist of the α, β, and γ isoforms, is licenced to treat cutaneous T-cell lymphoma.

The hypothesis, that we tested in this study, was that bexarotene would promote remyelination in demyelinated lesions in people living with relapsing remitting MS.

We conducted a two-centre, phase II clinical trial to determine the safety, tolerability and efficacy of bexarotene to promote remyelination of demyelinated lesions in people with relapsing remitting MS, using an innovative lesional MRI MTR outcome as well as visual evoked potentials.

Methods

Study design and participants

The Cambridge Centre for Myelin Repair Trial Number One (CCMR One) was a randomised, double-blind, placebo-controlled, parallel-group, phase II study conducted at the Cambridge University Hospitals NHS Foundation Trust and the University of Edinburgh Anne Rowling Regenerative Neurology Clinic. We recruited participants that had relapsing remitting MS, were aged 18-50 years, had an Expanded Disability Status Scale (EDSS) score of ≤6.0 and had ≥ 5 T2 hyperintense MS lesions on MRI. In order to minimise any confounding effect on the MRI endpoints of heterogenous disease-modifying therapies, only participants who had been receiving dimethyl fumarate – which has been shown to have no statistically significant effect on MTR[403] – for at least 6 months were selected, and this was continued on trial. Participants were ineligible if they had ever received a high-efficacy disease modifying treatment, had a history of pancreatitis, fasting triglycerides >2.3 mmol/L, uncontrolled thyroid disease, or excessive alcohol consumption. Amendments to eligibility criteria were recommended by the trial steering committee during the trial, additionally excluding those with significant cardiovascular disease or lymphopaenia (<0.7 x 10^9/L within 6 months of screening) in view of adverse events (AEs) observed in early trial participants.

The study was undertaken in accordance with the International Conference on Harmonisation Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki, registered with the ISRCTN (14265371) and was approved by London Westminster NRES Committee (15/LO/0108). All participants gave written informed consent.

Randomisation and masking

A web-based system (Tenelea, https://www.aleaclinical.eu/), run by an independent statistician, was used to randomise participants (1:1) by probability-weighted minimisation using four binary factors (age (≤ 40, > 40 years), gender, EDSS (≤ 4.0, > 4.0), and treatment centre), to a pack of indistinguishable over-encapsulated capsules of the investigational medicinal product (IMP). Participants and investigators were masked to treatment allocation. MRI scans and VEPs were labelled with secondary codes that did not identify the trial participant and were analysed at the end of the study; the MRI scans were analysed by Dr Brown, and the VEPs by myself. All endpoint data were locked before the treatment allocation code was broken by the trial statistician.

Procedures

The investigational medicinal product (IMP) was unmarked capsules of 75 mg bexarotene (Targretin®; Eisai Ltd) or placebo, provided by the Royal Free Hospital Pharmacy Manufacturing Unit, dosed at 300 mg/m^2 body surface area per day, rounded down to the nearest available number of whole (75 mg) capsules, not exceeding 750 mg per day. We saw participants weekly for one month then monthly for five months and finally at month 9. At each visit, safety blood tests included full blood count, creatinine, transaminases, fasting triglycerides, cholesterol and thyroid profile. In the event of hypertriglyceridaemia ≥10 mmol/L, fenofibrate 200 mg per day was commenced. If serum free thyroxine (FT4) fell below the lower reference limit, we prescribed levothyroxine 50 to 100 mcg and, when needed, increased the dose until FT4 normalised. Fenofibrate and thyroxine were stopped, per protocol, at month 6 with the IMP. If a participant developed neutropenia (<1.0 x10^9/L), we reduced IMP doses to 200 mg/m^2 and, if persistent, to 100 mg/m^2.

MRI scans were performed at baseline and 6 months using Siemens 3T Prismafit scanners (Siemens, Erlangen, Germany) with 20-channel head-neck coils at each site. Each scan included interleaved 3D magnetisation transfer imaging (for calculation of MTR maps), 3DT1 (for volumetric measures and segmentation), pre- and post-gadolinium T1 (for identification of enhancing lesions), interleaved proton-

density/T2-weighted scans (for identification and contouring of T2 hyperintense lesions) and fluid-attenuated-inversion recovery (FLAIR, for lesion identification). Lesion identification, contouring and checking were performed by blinded observers at the Queen Square MS centre. These baseline lesion masks were overlaid on the follow-up scans to ensure that the same tissue was examined at both timepoints (though did not accommodate dynamic effects from shrinking or expanding lesions). Lesions were automatically classified by location using the brain parcellation from the volumetric T1 scan.

Monocular full-field pattern-reversal visual potentials (VEPs) were performed at baseline and 6 months with check size 60-min of arc using a Nicolet Viking Select System (Natus Neurology Inc, USA) in Edinburgh and a Synergy System (Optima Medical Ltd, UK) in Cambridge. At least 100 stimuli were averaged per recording, and at least 2, but up to 4, recordings were taken from each eye at each visit. Using the Cambridge system, VEP recordings were extracted from their native software, anonymised by two members of the trial team, and exported into the Origin graphical application. From here I assessed each signal for quality control, removing those affected by muscle artefact or noise, while blinded to participant identity and treatment allocation. I was then able to extract the N75, P100 and N145 latencies for each waveform; the amplitude of the VEP was taken as the maximal N75-P100 peak-trough measure in μV and the values from repeated waveforms were averaged (Figure 2.1). The equivalent process was not possible technically with the Edinburgh electrophysiology equipment and instead anonymised reports were provided of the signal distributions of each VEP. This enabled me, in a blinded fashion, quality control each VEP by a similar manner of visual inspection.

The Expanded Disability Status Scale (EDSS) was assessed by a single clinician at each centre, blinded to all other assessments. Visual acuity was measured as the logarithm of the minimum angle of resolution (logMAR) for each corrected eye at a 100% contrast level.

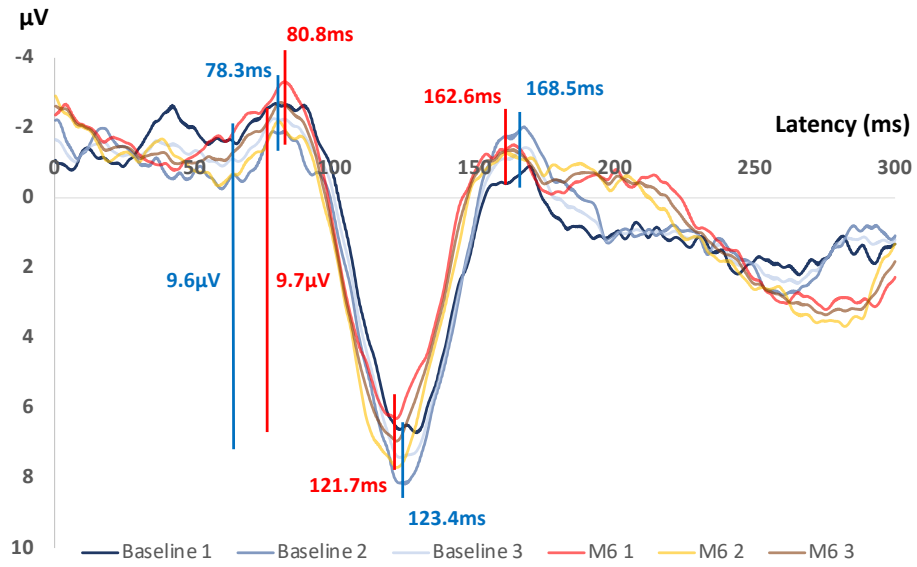


Figure 2.1. Representative averaged VEP signals from the right eye of a participant from CCMR One. At each visit, baseline and month 6, 3 recordings were taken, (blue traces at baseline, red/yellow at M6). These were then screened for quality control, and latencies and amplitude measures taken for the N75, P100, and N145 of each. These were averaged across a visit, to generate the values as shown; this participant had an improvement in P100 latency of 1.7 ms. (This participant reported no history of acute optic neuritis in this eye and was allocated to the placebo arm.)

Outcomes

The safety outcomes were adverse events and withdrawals attributable to bexarotene. I organised the AEs according to the MedDRA (the Medical Dictionary for Regulatory Activities) coding system. The primary efficacy outcome was the patient-level change in mean lesional MTR between baseline and month 6 for those lesions whose MTR was below the within-patient median at baseline. Prespecified exploratory lesion-level MRI analyses examined whether subgroups of lesions might better detect a treatment effect and included comparing treatment differences in mean lesional MTR (i) for lesions whose MTR was above versus below the within-cohort median and (ii) in different brain regions.

The main electrophysiological outcome for the trial were changes in P100 latency using full-field, pattern-reversal, VEPs, with separate analyses for all eyes and for those eyes with a baseline latency >118 ms (which are considered to be prolonged), and those with a past history of optic neuritis, with a per-protocol analysis pre-specified if treatment non-adherence was greater than 10%. I similarly assessed the changes in the N75 and N145 latencies and change in the N75-P100 amplitude.

Power calculation

Our trial used a novel primary efficacy endpoint, so could not draw on previous trial data for estimates of treatment effect. The rationale for our power calculations is described elsewhere.[329] In brief, we previously observed a difference between mean MTR of normal-appearing white matter (NAWM) and MS lesions of 5.92 pu. We assumed that only half of lesions would be amenable to remyelination and so estimated that a 100% treatment effect would be 0.5 × 5.92 = 2.96 pu. We chose a sample size sufficient to detect a 40% treatment effect (corresponding to a difference of 1.18 pu). The power of the baseline adjusted (ANCOVA) comparison method is dependent also on the correlation coefficient between MTR values at baseline and follow-up. A correlation of 0.73 was observed over a 12 month interval in the pilot data;[329] using a conservative correlation of 0.7 (since a higher correlation would be expected over six months), a sample size of 21 in each group is sufficient to detect the 40% treatment effect with 80% power at 5% significance. We chose 25 per group to allow up to 15% dropout.

Statistical analysis

The primary efficacy outcome, mean within-patient submedian lesion MTR, was chosen to guarantee that each patient would contribute lesions: those below the patient-specific lesion median MTR; using an all-lesion threshold instead might have resulted in some patients not contributing to the primary outcome. Treatment effect was estimated using multiple regression of the outcome measure on a group indicator with the following prespecified trial covariates: the baseline value of the outcome measure and the four binary minimisation factors: age (≤ 40 /> 40 years), gender, trial centre/scanner (London/Edinburgh) and EDSS (≤ 4.0/> 4.0 score). The

lesion-level MTR analyses used linear mixed models for lesions nested within patients, with patient random intercepts; these models regressed lesion MTR on the same prespecified covariates but with lesion-subgroup interaction terms to estimate lesion-subgroup specific treatment differences and test for variation between these differences. These interaction tests were relatively highly powered because a strong within-patient component increased precision, whereas treatment difference estimation was entirely between-patient. Although lesion-level analyses are more flexible and powerful, they are vulnerable to selection bias since patients not lesions are randomised, so the patient-level comparison was designated primary.

Similar mixed models were also used for the VEP analyses, but with eyes nested within patients. In exploratory analyses, outside of the main trial results, I performed a joint test of the 6 latency points (3 latencies x 2 eyes) by the fitting a multivariate model which regresses all six outcomes simultaneously, taking into account correlation between the outcomes: the more correlated the outcomes, the less weight of evidence is given to a number of similar treatment effects. (In an extreme case where essentially the same measure is compared six times, with almost perfect correlation between the six measures, there is no additional evidence, provided by the joint test of the six virtually identical measures, compared to that provided by a single comparison.) This tests the null hypothesis that there is in truth no active vs placebo difference in any of the six comparisons. Such a null hypothesis is less likely to be true, and its rejection more likely to be interpretable, when most or all results fall in the same direction. Then if this null hypothesis is rejected with a single p-value, it provides overall evidence that there is a true active vs placebo difference, in the prevalent direction, in at least one, and of course possibly in more than one, of the six separate comparisons; and if this null hypothesis is rejected, it inspires confidence that if, for example, only some of the six are statistically significant at the 5% level, these are not just spurious false positives. Joint tests of this sort thus provide a more informed alternative to “corrections” for multiple comparisons which do not quantify the similarity of the outcomes tested.

For EDSS, a corresponding multiple regression was checked using a non-parametric bias-corrected and accelerated bootstrap[404] with 1000 replicates. For both regression and mixed models, residuals were examined for departures from normality and homoscedasticity, and satisfied assumptions. Statistical significance refers to two-sided $p<0.05$.

Results

Between Jan 17th, 2017 and May 17th, 2019, we randomly assigned 52 patients to receive 6 months of bexarotene (n=26) or placebo (n=26; Figure 2.2). Two participants randomised to placebo were withdrawn before receiving the IMP: one was unable to tolerate the baseline MRI, while another had a new lesion on their baseline scan requiring treatment escalation from dimethyl fumarate. One participant withdrew consent for personal reasons at month 2. The remaining patients (31 at Cambridge and 18 at Edinburgh) attended all trial visits and completed the trial (Figure 2.2) and their baseline characteristics are included in Table 2.1.

Safety

In performing the safety analysis, I demonstrated that participants receiving bexarotene experienced a mean of 6.1 adverse events (compared to 1.6 on placebo). The study drug was discontinued in 5 (19%) and 2 (8%) participants in the bexarotene and placebo groups respectively due to adverse events (Table 2.2).

All 26 (100%) bexarotene-treated participants developed central hypothyroidism (Figure 2.3). 24 of these required levothyroxine; two chose to withdraw from bexarotene because of a skin rash before levothyroxine could be started. 24 bexarotene participants (92%) developed raised triglyceride levels; six of these reached ≥10 mmol/L and were commenced on fenofibrate. The median highest triglyceride level, per participant, was 4.85 (IQR 4.10, 10.02) mmol/L on bexarotene compared to 1.25 (IQR 0.98, 1.83) mmol/L on placebo. Neutropenia ($<1.0 \times 10^9$/L) occurred in 10 (38%) patients in the bexarotene group, requiring dose reductions in all, and treatment withdrawal in one. Skin reactions and headaches occurred more

commonly in the bexarotene group (18 (69%) vs 2 (8%) and 14 (54%) vs 8 (33%) respectively). One participant on bexarotene, without vascular risk factors and a peak triglyceride level of 4.2 mmol/L, had an asymptomatic cerebellar infarct noted on the month 6 scan. By month 9, at least three months after discontinuing bexarotene, all participants' thyroid, lipid and neutrophil counts were normal. There were no pancreatitis or cardiovascular events.

MRI outcomes

All MRI scans were of sufficient quality to be included in the efficacy analyses, and 3170 T2 hyperintense lesions were identified (1613 white matter (WM) lesions, 106 grey matter (GM) lesions and 1451 mixed GM and WM lesions). There were too few enhancing lesions at baseline (single lesions in 3 patients, Table 2.1) or new T2 hyperintense lesions at follow-up (7 lesions in 5 patients) to warrant further analysis.

The primary efficacy endpoint of the intention to treat (ITT) population showed no evidence of treatment effect: the bexarotene – placebo adjusted difference in mean within-patient submedian lesion MTR change was 0.16 (95% CI -0.39, 0.71) pu, p=0.554; Table 2.3, Figure 2.4A. The upper limit of the confidence interval is well below the target 1.18 pu which the trial was powered to detect. In exploratory analyses, when the median MTR was defined for all lesions in the ITT population, bexarotene had no effect on supramedian lesions (-0.04 (95% CI -0.52, 0.43) pu, p=0.854) and a non-statistically significant increase in MTR for submedian lesions (0.30 (95% CI -0.18, 0.78) pu, p=0.223, Table 2.3, Figure 2.4B). However, an interaction term comparing the treatment group differences between submedian and supramedian lesions was highly statistically significant (p=0.007), suggesting a variation in treatment effect depending on the baseline lesional MTR.

When lesions were subdivided by location (Table 2.3), statistically significant treatment effects were seen in the ITT population within cortical GM lesions (bexarotene-placebo adjusted mean difference 1.00 (95% CI 0.17, 1.83) pu, p=0.023), deep GM lesions (1.93 (95% CI 0.28, 3.59) pu, p=0.027) and brainstem lesions (1.75 (95% CI 0.86, 2.63) pu, p=0.0004), and the interaction test of variation

in treatment effects gave p<0.0001 (Figure 2.4C). A statistically significant treatment effect was seen in pure GM lesions (1.08 (95% CI 0.32, 1.83) pu, p=0.008) but not in pure WM lesions (0.10 (95% CI -0.38, 0.68) pu, p=0.568) (interaction test p=0.002).

	Bexarotene	Placebo
Total number of participants Cambridge, number (%) Edinburgh, number (%)	25 16 (64) 9 (36)	24 15 (63) 9 (37)
Age, years; mean (SD)	40.4 (6.2)	38 (6.8)
Sex Female Male	 15 10	 13 11
Disease duration, years; mean (SD)	11 (5.9)	8.4 (5.8)
Number of relapses in last 2 years; mean (SD)	0.4 (0.7)	0.9 (1.2)
EDSS step; median (quartiles)	2.5 (1.5, 3.5)	2.0 (1.5, 3.0)
Duration receiving dimethyl fumarate, years; median (quartiles)	2.2 (1.1, 3.2)	1.6 (0.9, 2.3)
MRI		
Within-patient number of T2 lesions; median (quartiles)	63 (51, 111)	43 (19.5, 66)
Within-patient size of T2 lesions, mm^2; median (quartiles)	90.6 (67.7, 146.0)	83.96 (55.4, 119.3)
Within-group total number of contrast-enhancing lesions at baseline	3	0
Within-patient lesional MTR, pu; mean (SD)	41.83 (2.03)	41.73 (2.08)
Within-patient brain parenchymal fraction; mean (SD)	0.74 (0.02)	0.75 (0.01)
VEP		
Total number of VEP recordings with sufficient quality for inclusion; number of eyes (number of patients)	42 (22)	44 (23)
Participants with history of ON (number of eyes)	11 (12)	14 (20)
Time since ON, years; median (quartiles)	12.2 (4.4, 15.5)	3.3 (1.6, 8.6)
VEP P100 latency, ms; mean (SD)	126.2 (18.6)	119.3 (18.1)

Table 2.1. Comparison of baseline variables between the two trial arms for all participants in the intention-to-treat analyses. One further participant randomised to bexarotene (who withdrew before month 2) and two further participants randomised to placebo (withdrawn before commencing the IMP) had no follow-up MRI or VEP so could not be included in the intention to treat analyses. EDSS: expanded disability status scale; MTR: magnetization transfer ratio; ON: optic neuritis; VEP: visual-evoked potential.

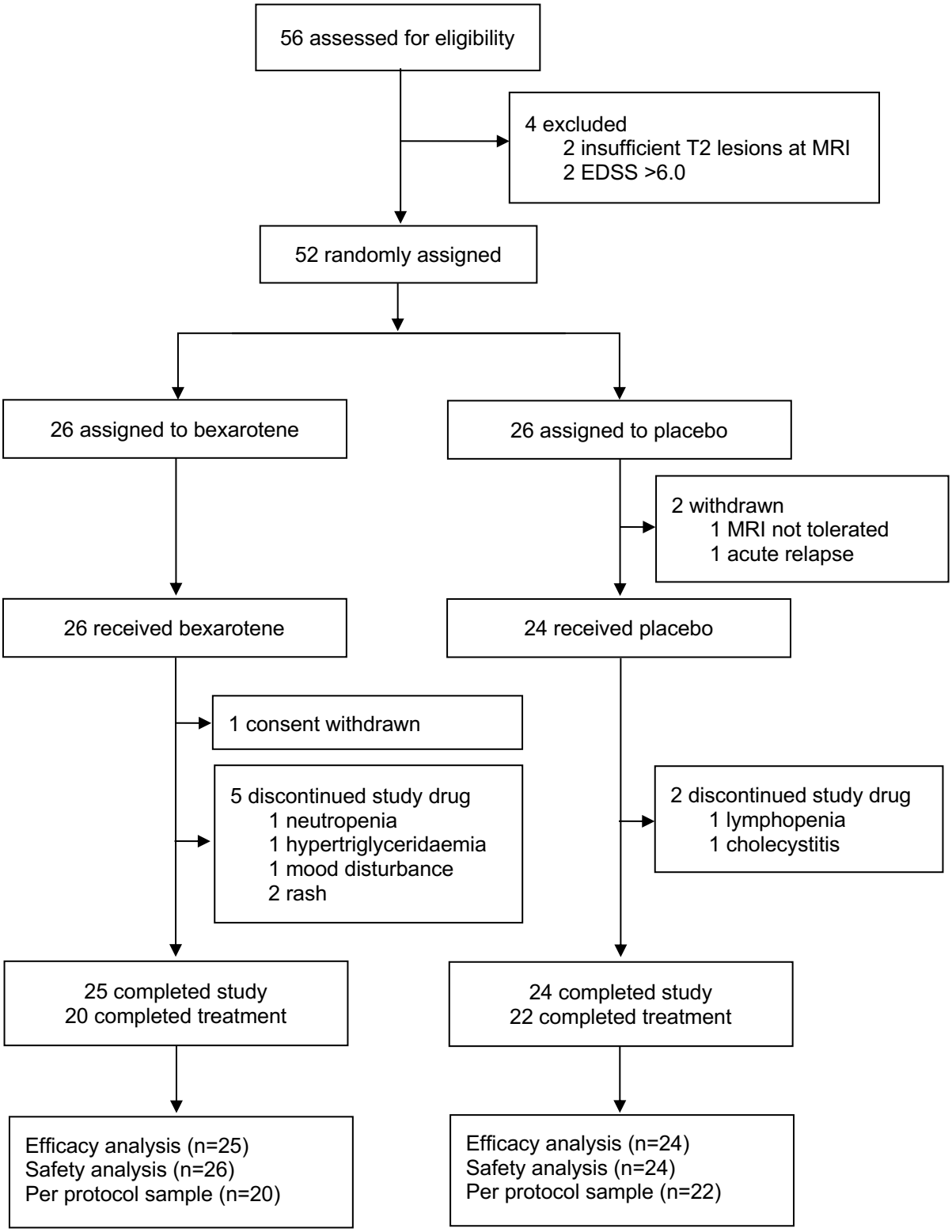


Figure 2.2. Trial design. EDSS: expanded disability status scale.

	Bexarotene (n=26)	Placebo (n=24)
All adverse events		
Number of adverse events (mean per person)	159 (6.12)	39 (1.63)
Number of participants with ≥1 adverse event (%)	26 (100%)	17 (71%)
Number of participants who discontinued study drug because of adverse event (%)	5 (19%)	2 (8%)
Serious adverse events		
Hospitalisation	0	1 (4%) *
Expected adverse events		
Metabolic and nutrition disorders		
Hypertriglyceridaemia	24 (92%)	0
Secondary (central) hypothyroidism	26 (100%)	0
Blood and lymphatic system disorders		
Neutropenia	10 (38%)	0
Lymphopenia	1 (4%)	1 (4%)
Nervous system disorders		
Headache	14 (54%)	8 (33%)
Skin and subcutaneous tissue disorders		
Rash	13 (50%)	1 (4%)
Pruritis	7 (27%)	0
Unexpected adverse events		
Nervous system disorders		
MS Relapse	1 (4%)	0
MS Pseudorelapse	1 (4%)	4 (17%)
Lhermitte's sign	1 (4%)	0
Cerebellar infarction	1 (4%)	0
Neuropathic pain	1 (4%)	1 (4%)
Muscle spasticity aggravated	1 (4%)	0
Dizziness	1 (4%)	0
Low mood	1 (4%)	0
Memory disturbance	0	1 (4%)
Skin and subcutaneous tissue disorders		
Skin desquamation	5 (19%)	0
Dry skin	4 (15%)	0
Acne	1 (4%)	0
Alopecia	1 (4%)	0
Facial flushing	0	2 (8%)
Dry eyes	1 (4%)	0
Infections and infestations		
Upper respiratory tract infection	2 (8%)	1 (4%)
Lower respiratory tract infection	1 (4%)	0
Urinary tract infection	2 (8%)	1 (4%)
Shingles	0	1 (4%)
Ear infection	1 (4%)	0
Coryzal symptoms	3 (12%)	4 (17%)
Sinusitis	0	1 (4%)

Gastrointestinal and hepatobiliary disorders		
Nausea	5 (19%)	0
Diarrhoea	4 (15%)	4 (17%)
Constipation	2 (8%)	0
Epigastric pain	1 (4%)	0
Dry lips	2 (8%)	0
Ulceration of mouth	2 (8%)	0
Cholecystitis	0	1 (4%)
Respiratory, thoracic and mediastinal disorders		
Cough	1 (4%)	1 (4%)
Shortness of breath	0	1 (4%)
Sore throat	1 (4%)	1 (4%)
Musculoskeletal and connective tissue disorders		
Stiffness joints	1 (4%)	1 (4%)
Myalgia	1 (4%)	0
Renal and urinary disorders		
Nocturia	2 (8%)	0
Urinary frequency	2 (8%)	0
Vascular disorders		
Epistaxis	1 (4%)	0
General disorders		
Fatigue	6 (23%)	4 (17%)
Investigations		
Transaminitis	3 (12%)	0
Weight loss	1 (4%)	0

Table 2.2. Adverse events in each of the two trial arms for participants who received at least one IMP dose. Unless otherwise stated, values are numbers of participants (%) with at least one event of the stated type. *One patient, on placebo, was hospitalised overnight for treatment of cholecystitis. Expected adverse effects of bexarotene, identified from the Summary of Product Characteristics.

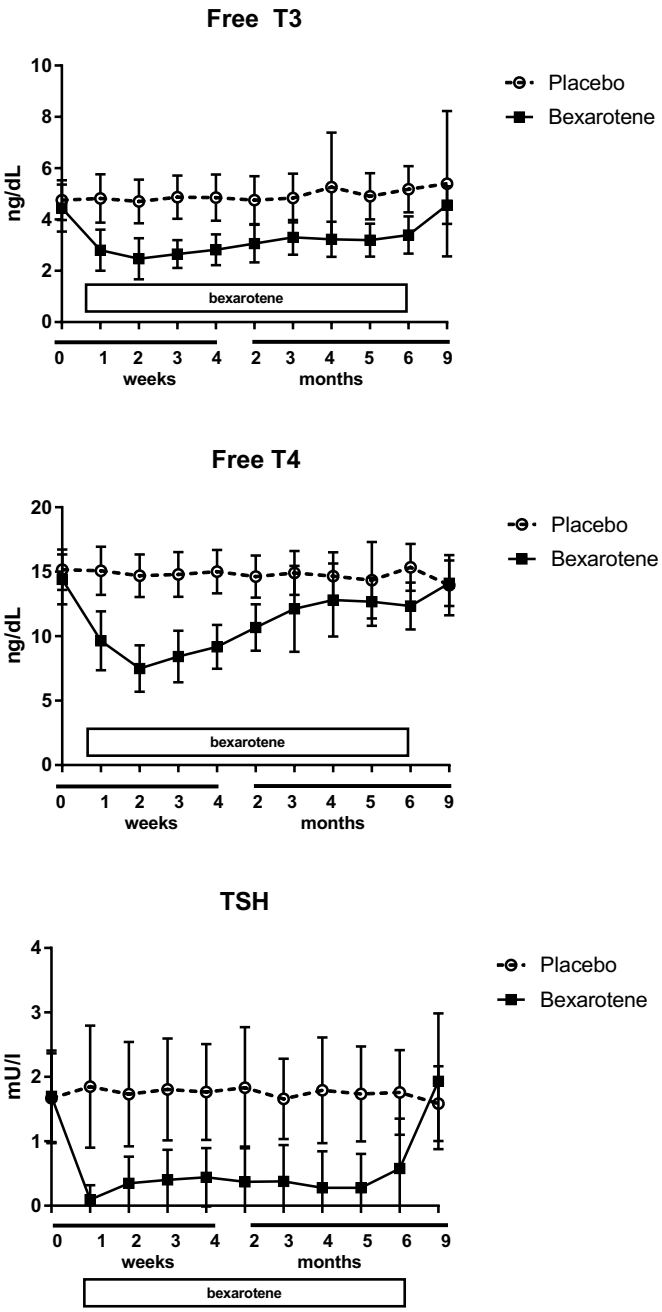


Figure 2.3. Variation in fasting blood levels of Free T3, Free T4 and thyroid stimulating hormone (TSH) in patients receiving placebo or bexarotene. Blood tests performed weekly (for the first month), then monthly until month 6 (when the study drug ceased) then finally at month 9.

	Bexarotene		Placebo		Bexarotene-placebo change	
Subgroup of lesions	**Patient* or lesion number**	**Unadjusted mean (SD) change in lesional MTR (pu)**	**Patient* or lesion number**	**Unadjusted mean (SD) change in lesional MTR (pu)**	**Adjusted bexarotene-placebo difference (95% CI)**	**p-value**
Patient submedian lesion mean**	25*	0.25 (0.98)	24*	0.09 (0.84)	0.16 (-0.39, 0.71)	0.554
Submedian lesions (defined by cohort-level median)	923	0.35 (2.09)	662	-0.07 (1.68)	0.30 (-0.18, 0.78)	0.223
Supramedian lesions (defined by cohort-level median)	1023	-0.31 (1.74)	562	-0.18 (1.51)	-0.04 (-0.52, 0.43)	0.854
Interaction test comparing treatment group differences between submedian and supramedian lesions						0.007
Periventricular lesions	205	-0.31 (1.70)	151	-0.18 (1.33)	-0.02 (-0.58, 0.55)	0.953
Deep WM lesions	593	-0.03 (1.72)	356	0.01 (1.39)	-0.06 (-0.56, 0.44)	0.810
Juxtacortical lesions	82	0.09 (1.71)	53	-0.16 (2.15)	0.29 (-0.44, 1.01)	0.441
Leucocortical lesions	650	0 (2.08)	393	-0.02 (1.62)	-0.04 (-0.54, 0.46)	0.867
CGM lesions	46	0.69 (2.58)	39	-0.42 (3.20)	1.00 (0.12, 1.75)	0.023
DGM lesions	7	0.49 (2.81)	9	-1.41 (1.25)	1.93 (0.28, 3.59)	0.027
Mixed DGM and WM lesions	217	0.10 (1.80)	158	-0.24 (1.43)	0.41 (-0.15, 0.97)	0.160
Brainstem lesions	64	0.24 (2.62)	24	-1.21 (1.59)	1.75 (0.86, 2.63)	0.0003
Cerebellar lesions	82	0.04 (2.28)	41	-0.31 (1.54)	-0.03 (-0.79, 0.74)	0.947
Interaction test comparing treatment group differences between lesion locations						<0.0001

Table 2.3. Trial MRI outcomes for intention to treat analysis. p values and CIs are for the adjusted (for baseline value and prespecified covariates) bexarotene – placebo differences. MTR analyses are at the lesion level (for the same patient numbers given in the first table row) unless stated as being at patient level. MTR: magnetization transfer ratio; pu: percentage units; WM: white matter; CGM: cortical grey matter; DGM: deep grey matter. **primary efficacy outcome measure.

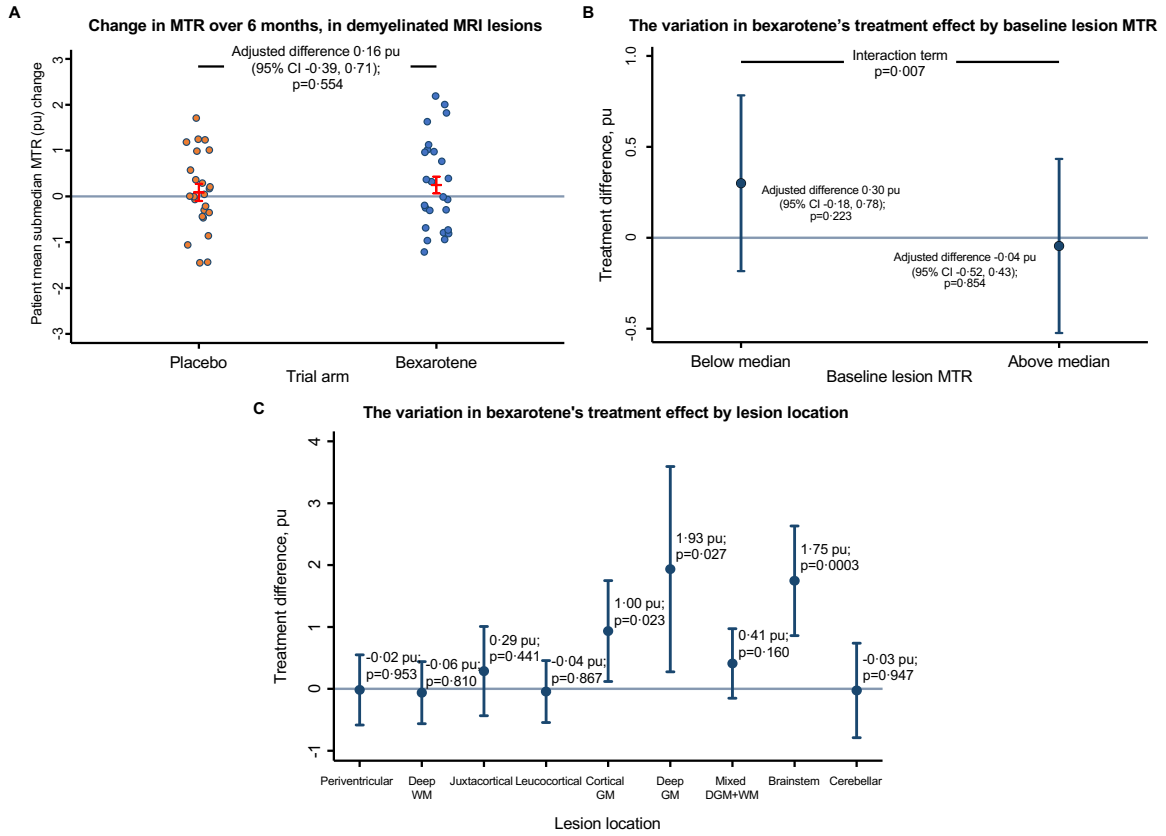


Figure 2.4. MRI outcomes. A: The change between month 6 and baseline in patient mean submedian lesional MTR by trial group. Bars are standard errors around the unadjusted group mean changes. B: The active-placebo adjusted differences in lesional MTR change, subdivided by lesion baseline MTR relative to the lesion sample median. Bars are 95% confidence intervals. C: The active-placebo adjusted differences in lesional MTR change, subdivided by lesion location. Bars are 95% confidence intervals. Pu: percentage unit; GM: grey matter; DGM: deep grey matter; WM: white matter.

Electrophysiology outcomes

86 out of 98 (88%) VEP recordings were of sufficient quality to be analysed. 27 of these eyes had previously been affected by an episode of clinical acute optic neuritis; six having occurred within 2 years of baseline, a further nine between 2 and 5 years of baseline and twelve 5 years or more from baseline. One episode of acute optic neuritis occurred on the trial in a participant in the bexarotene group. We debated this diagnosis as it consisted of a painless reduction in visual acuity in an eye previously affected by ON, but as it lasted 3 weeks, in the absence of another illness, and was associated with a reduction in visual acuity, it was classified as a relapse; this participant was not, however, treated with steroids.

In our prespecified analysis of eyes with baseline latency of >118 ms (29 bexarotene, 22 placebo), the adjusted bexarotene-placebo difference was -4.06 ms (95% CI -7.68, -0.44) p=0.028; Table 2.4, Figure 2.5. This difference remained statistically significant after excluding eyes affected by clinical optic neuritis within 5 years (adjusted latency difference was –4.75 ms (95% CI -8.80, -0.71), p=0.032 in an intention to treat (ITT) analysis, and -6.54 ms (95% CI, -10.62, -2.47), p=0.006 in the per protocol (PP) group). Excluding the eye that was likely affected by AON on trial made no material difference to these results.

When all eyes were included (42 bexarotene and 44 placebo) there was a borderline statistically significant treatment effect in the ITT analysis (adjusted difference -2.85 ms (95% CI -5.75, 0.05), p=0.054), but in the PP analysis a larger statistically significant adjusted difference (-4.02 ms (95% CI -7.27, -0.76), p=0.015)) was seen; Figure 2.5.

	Bexarotene		Placebo		Bexarotene-placebo change	
	Number of eyes (patient)	**Unadjusted latency, ms; mean (SD) change**	**Number of eyes (patient)**	**Unadjusted latency, ms; mean (SD) change**	**Adjusted difference (95% CI)**	**p-value**
All eyes	42 (22)	-2.00 (6.20)	44 (23)	0.70 (4.71)	-2.85 (-5.75, 0.05)	0.054
Eyes with P100 ≤118ms at baseline	13 (7)	1.27 (2.75)	22 (13)	1.01 (5.35)	-0.24 (-4.64, 4.16)	0.916
Eyes with P100 >118ms at baseline	29 (16)	-3.46 (6.78)	22 (12)	0.40 (4.08)	-4.06 (-7.68, -0.44)	0.028
Eyes with P100 >118ms at baseline and no ON in previous 5 years	26 (15)	-3.87 (6.97)	17 (11)	0.08 (4.12)	-4.75 (-8.80, -0.71)	0.032

Table 2.4. Trial electrophysiology outcomes for intention to treat analysis. p values and CIs are for the adjusted (for baseline value and prespecified covariates) bexarotene – placebo differences. One participant in the bexarotene group, and two in the placebo group, contributed one eye to each of the ≤118 ms and >118 ms subgroups. VEP: visual evoked potential; ON: optic neuritis.

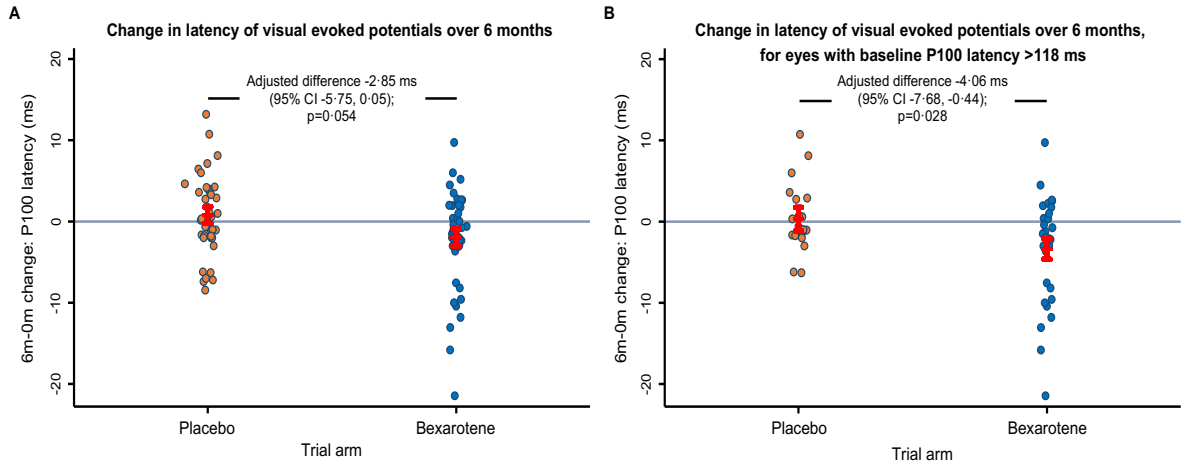


Figure 2.5. Electrophysiological outcomes. A: the change in P100 latency between month 6 and baseline for all eyes subdivided by trial group. B: the change in P100 latency between month 6 and baseline for those eyes with a delayed (>118 ms) latency at baseline subdivided by trial group. Bars are standard errors around the unadjusted group mean changes.

In additional analyses I conducted outside of the main trial outcomes, for N75-P100 amplitude, the adjusted bexarotene-placebo difference was 0.16 μV, (95% CI -1.12, 1.43) p=0.807, with a larger but still non-statistically significant improvement among those eyes that had evident optic neuropathy at baseline (adjusted difference 0.64 μV (95% CI -1.09, 2.38) p=0.467). For N75 latency, there was a statistically significant adjusted bexarotene-placebo difference of -3.46 ms (95% CI -6.69, -0.23) p=0.036, which was more significant still in the optic neuropathy group (adjusted difference -5.92 ms (95% CI -10.20, -1.63) p=0.007). Finally, for N145 latency, the adjusted bexarotene-placebo difference was -3.18 ms (95% CI -7.04, 0.68) p=0.106, while there was a borderline statistically significant treatment effect in the optic neuropathy group (adjusted difference -5.11 ms (95% CI -10.23, 0.02) p=0.051).

Overall evidence of a treatment effect on latencies was given by the joint test of the six latency measures. Five of the six outcomes showed a more negative bexarotene than placebo latency change; N145 right eye latency was non-statistically significantly higher in bexarotene than placebo. Jointly testing the six comparisons gave p=0.0012, providing with a single p-value evidence at the 1% level of a treatment effect in one or more of the six measures. This provides reassurance that statistically significant improvements on one part of the distribution are not just spurious false positives.

Clinical efficacy

This trial was not powered to detect a treatment effect on disability and none was seen on change in EDSS from baseline to 6 months (adjusted bexarotene-placebo difference 0.33 (-0.10, 0.76), p=0.134). In a retrospective analysis of the high-contrast visual acuities performed during the EDSS assessments, there was also no treatment effect (adjusted bexarotene-placebo difference in logarithm of minimum angle of resolution (logMAR) 0.03 (-0.03, 0.08), p=0.339).

Discussion

Bexarotene was poorly tolerated and the primary efficacy objective, using an MRI endpoint untested in previous trials, was not met. Nonetheless converging evidence from several other MRI and electrophysiological outcomes, in a trial not powered to detect a treatment difference with these outcomes, suggests that bexarotene has a small biological effect to promote remyelination in some demyelinated lesions in the brains of people with MS. This aligns with the preclinical finding that RXR-γ agonists enhance remyelination.[291]

The electrophysiology data suggest that bexarotene has restored conduction in demyelinated pathways. These effects were numerically greater, and statistically significant, when excluding eyes with a normal baseline P100 latency (defined as ≤118 ms). The results were clearer still in the per-protocol analyses; it is perhaps significant that 3 of the 5 participants that stopped taking bexarotene, received the drug for less than one month, which might explain the larger effect in the PP group despite the smaller sample size. I cannot exclude a direct action of bexarotene on demyelinated axons to promote conduction of the action potential, although none has been described previously, and submit that this most likely reflects remyelination. Hypothyroidism, experienced by most people on bexarotene would, if anything, prolong the P100 latency,[405] and while other retinoids have not been reported to affect VEPs, they prolong latencies of the auditory evoked potential.[406]

The MRI data suggests that multiple sclerosis lesions are heterogeneous in their response to retinoid X receptor agonists. Grey matter plaques showed greater remyelination than those in white matter, which is consistent with the pathology literature.[270,271,407] The higher grey matter content of the brainstem may explain the greater treatment effect seen in lesions there, but segmentation of the brainstem into grey and white matter to confirm this was not possible technically. Additionally, there was a trend to an improvement in the MTR of submedian lesions (defined at the cohort level), while there was unsurprisingly no discernible treatment effect in supramedian lesions. The significant interaction term between these treatment

differences, however, suggests that remyelination is dependent on baseline MTR, and that there is a small biological effect of bexarotene.

Despite these effects, we do not recommend the use of bexarotene in people with MS. It caused secondary (central) hypothyroidism in all patients, raised triglycerides in 92%, headache in 54%, rash in 50% and neutropenia in 38%. It is possible that the reason rates of hypothyroidism and raised triglycerides exceeded those seen when used in cutaneous T cell lymphoma (29% and 71% respectively)[408] is an interaction with dimethyl fumarate, whose effects on nrf2 transcription may additionally have been suppressed by bexarotene.[409] More selective RXR-γ agonists, which are not currently available, would reduce the adverse effects mediated by agonism of the RXR-α and RXR-ß pathways,[292] although thyroid dysfunction would remain a potential adverse effect of RXR-γ agonists.[410]

This is the first clinical trial that has shown remyelination on both MRI and electrophysiological measures (reviewed by Lubetzki[400] and Cunniffe[401]). Mesenchymal stem cells led to improvements in VEP latency and visual acuity but not MTR.[302] Clemastine reduced VEP latency in eyes with chronic stable optic neuropathy but had no impact on MRI outcomes.[277] Opicinumab reduced VEP latency in acute optic neuritis in a per protocol analysis, but had no effect on MRI measures.[285] MTR increases were reported with an H3 receptor antagonist (in gadolinium-enhancing lesions).[290]

Perhaps one of the legacies of this trial will be the lessons learned for the design of future trials examining remyelination in MS. This study demonstrated that MS lesions are heterogeneous in their capacity for remyelination in response to RXR agonists with greatest remyelination in lesions that were more demyelinated at baseline and those located in grey matter. Enhanced remyelination of cortical grey matter neurons may also have contributed to the improved visual evoked potential, since less than half the variance of VEP latency can be attributed to MRI lesions within the visual tract.[411] At 3T, FLAIR detects less than 7% of pure CGM lesions at post-mortem; it identifies no intracortical or purely subpial lesions.[324] The cortical GM lesion results

may therefore not be generalisable to all cortical lesions. Future phase II remyelination trials should therefore use both VEP and MRI outcome measures sensitive to grey matter lesions.[323] The advantage of MRI lesion-level analyses, enabling relatively powerful formal treatment effect comparisons in different lesion types, is offset by the fact that patients, not lesions, are randomised, the latter being potentially vulnerable to selection bias. The exploratory lesion level results here should therefore be considered hypothesis-generating. But this study does suggest that focusing patient-level analyses on certain lesion types may be promising.

Limitations of this study are that it was not powered to detect a treatment difference with the exploratory outcomes. Also, although our trial was based on preclinical work showing RXR-γ agonists' direct effect on OPCs,[291] other mechanisms may be at play. Bexarotene may also have enhanced remyelination indirectly by increasing phagocytosis of myelin debris,[412] which inhibits OPC differentiation,[28] through the RXR-α pathway. We cannot exclude the possibility that thyroxine, used to treat 24 patients' hypothyroidism in the bexarotene arm, promoted remyelination,[413] although patients' T3 and T4 levels never rose above pre-treatment levels (Figure 2.3). Nevertheless, our data, together with other studies using therapies that target OPC differentiation,[277,285,286] suggest this is a viable approach to promote remyelination in MS.

Trials of remyelinating treatments mark the beginning of a new phase in the treatment of MS, following success in suppressing the inflammatory component of MS. Although bexarotene is unlikely to become a future treatment of MS because of its serious adverse effects, this trial identifies a potential new strategy, RXR-γ agonism, and informs future designs, for remyelinating trials.

Chapter 3: The impact of age on the remyelinating effect of bexarotene

A post-hoc analysis of the trial data from CCMR One

Abstract

Remyelination becomes less efficient with advancing age in animal models, but the same has been more challenging to demonstrate in people with multiple sclerosis. While the histopathological literature indicates that extensive remyelination is achievable in elderly patients, remyelination appears to proceed more efficiently in younger people with MS. In phase II remyelination trials to date, few have interrogated the effect of age on treatment response.

To that end, I conducted an exploratory analysis of the electrophysiological and magnetic resonance imaging markers of remyelination from the Cambridge Centre for Myelin Repair One (CCMR One) trial (ISRCTN14265371). In doing so I have shown an age-dependent treatment response amongst patients receiving the retinoid-X receptor agonist bexarotene. For eyes with chronic optic neuropathy (baseline latency >118 ms), bexarotene shortened the full-field visual evoked potential P100 latency maximally in younger patients, with the effect diminishing by 0.45 ms per year of age. Similarly, age-dependent remyelination was seen in deep grey matter lesions.

I conclude that interventions focussed on reversing hallmarks of ageing in OPCs – for example rejuvenating drugs, dietary interventions or gene editing – are likely to be needed to maximise the effects of drugs with a pro-differentiating mechanism of action, such as bexarotene and clemastine.

Contribution statement

As described in the previous chapter, I was an evaluating physician and sub-investigator on this trial, involved in all aspects of the data collection except for the analysis of the trial MRI brain scans. I conducted the analysis of these data, with technical support from Dr Christopher McMurran. I am grateful for advice from Dr Daniel Altmann, who reviewed our results and advised on the analysis plan.

Background

As discussed in the introduction, in animal models remyelination becomes inefficient with advancing age, due to both an intrinsic decline in oligodendrocyte progenitor cells (OPCs)[100] and adverse changes in their environment.[268,414] As a result, much preclinical research is focused on interventions to reverse hallmarks of ageing in OPCs, by, for example, rejuvenating drugs, dietary interventions or gene editing and thereby reverse age-related decline in remyelination (reviewed by Neumann et al.[415]).

Despite this, direct evidence for the same age-related decline in people with MS has been hindered by the challenges of measuring human remyelination in unbiased populations at meaningful timepoints. Pathological studies of remyelination have demonstrated that, whilst younger lesions are better remyelinated than chronic lesions,[98] extensive remyelination can be observed in the brains of elderly patients.[98,214,270] However, post-mortem studies can be confounded by survivorship bias, whereby patients that die in old-age are likely to be those with a more quiescent disease course, who accumulate remyelinated lesions throughout their lifespan: it is impossible to know when lesions that have undergone remyelination arose or how long they took to repair. Some of these drawbacks are overcome by longitudinal imaging studies, which hint at an age-dependent repair process. For example, older patients are more likely to develop large lesions with a persistent pro-inflammatory rim, associated with minimal remyelination at post-mortem.[97] Clinical studies of disability accumulation are also consistent with an age-dependent repair process: older onset of MS is associated with faster development of disability,[416] disability milestones tend to be reached at consistent ages regardless of prior disease course[256] and less recovery is seen following relapses in older patients.[417] These are important outcomes for patients, but clearly the accumulation of disability in people with MS is attributable to many factors in addition to remyelination failure.

I hypothesised that the response to bexarotene would decline with advancing age. I therefore investigated the effect of patient age on the electrophysiological and MRI

markers of remyelination in a post-hoc analysis of the CCMR One trial (ISRCTN14265371, Chapter 2).

Methods

The full protocol and results from the CCMR One trial are discussed in Chapter 2. Briefly, patients with relapsing remitting MS were randomised to receive six months of either bexarotene (n=26) or placebo (n=26), with remyelination in the visual pathway assessed using full-field visual evoked potential (VEP) latencies,[418] and lesion magnetisation transfer ratio (MTR) used to quantify remyelination in brain lesions.[319] The age range of patients receiving bexarotene was 29 to 49 (mean 40.4) while those receiving placebo was 25 to 49 (mean 38.0). Data was analysed by intention to treat.

Statistical analysis was performed in R. For VEP analysis, the effect of patient age was estimated using linear mixed models for eyes nested within patients, with patient random intercepts. The change in P100 latency was regressed on an interaction between age, treatment group and baseline value (≤ 118 / >118 ms), as well as three binary minimisation factors: EDSS (≤ 4.0 / > 4.0), gender and trial centre. Residuals were examined for departures from normality and homoscedasticity, and satisfied assumptions. For MRI analysis, lesions were nested within patients, with patient random intercepts. Change in whole lesion MTR was regressed on an interaction between age, treatment group and lesion location, as well as baseline MTR and the three minimisation factors. Residuals for the MTR models were non-normal, so confidence intervals were verified using a bootstrap approach with 500 replicates. Differences between treatment and control groups as a function of age were calculated using the Johnson-Neyman technique.

Results

Previous work has identified the most robust effects of pro-remyelinating therapies on VEP amongst eyes with a prolonged baseline P100 latency (>118 ms). Analysing these eyes from participants of CCMR One, I found that bexarotene shortened the P100 latency maximally in younger patients (Figure 3.1A). With increasing age, the P100 improvement amongst patients on bexarotene diminished by 0.45 ms/year (95% CI 0.03, 0.88; p=0.044). Compared to patients on placebo, bexarotene significantly improved P100 latency only in patients below the age of 42 ($\alpha = 0.05$, Figure 3.1B). The age-dependence of P100 improvement in the bexarotene group was magnified when eyes affected by optic neuritis during the trial or in the previous 5 years were excluded (0.64 (95% CI 0.24, 1.04) ms/year, p=0.004).

To investigate whether this age-dependence may instead represent an effect of disease duration, I replaced the age term in the model with disease duration (years since symptom onset). Unlike age, disease duration did not significantly modify the P100 improvement in the bexarotene group (0.18 (95% CI -0.26, 0.62) ms/year; p=0.43)).

I next explored whether an age-dependent effect of bexarotene was also seen on MRI, by analysing change in lesion MTR as a marker of remyelination. In the CCMR One trial, bexarotene was found to have maximal remyelinating activity amongst lesions in the deep grey matter, cortical grey matter and brainstem (Chapter 2). Focusing on these areas, I identified a significant age-related attenuation in MTR improvement amongst deep grey matter lesions (-0.34 (95% CI -0.64, -0.04) pu/year, p = 0.028, Figure 3.2A-B). Compared to patients on placebo, bexarotene significantly improved deep grey matter lesion MTR only in patients younger than 43 ($\alpha = 0.05$, Figure 3.2C). Lesion MTR improvements in cortical grey matter (0.08 (95% CI -0.01, 0.18) pu/year, p=0.09) and brainstem (-0.01 (95% CI -0.10, 0.07) pu/year, p=0.73) did not significantly depend on age in the bexarotene group, and none of these regions showed significant age-dependent remyelination in the placebo group.

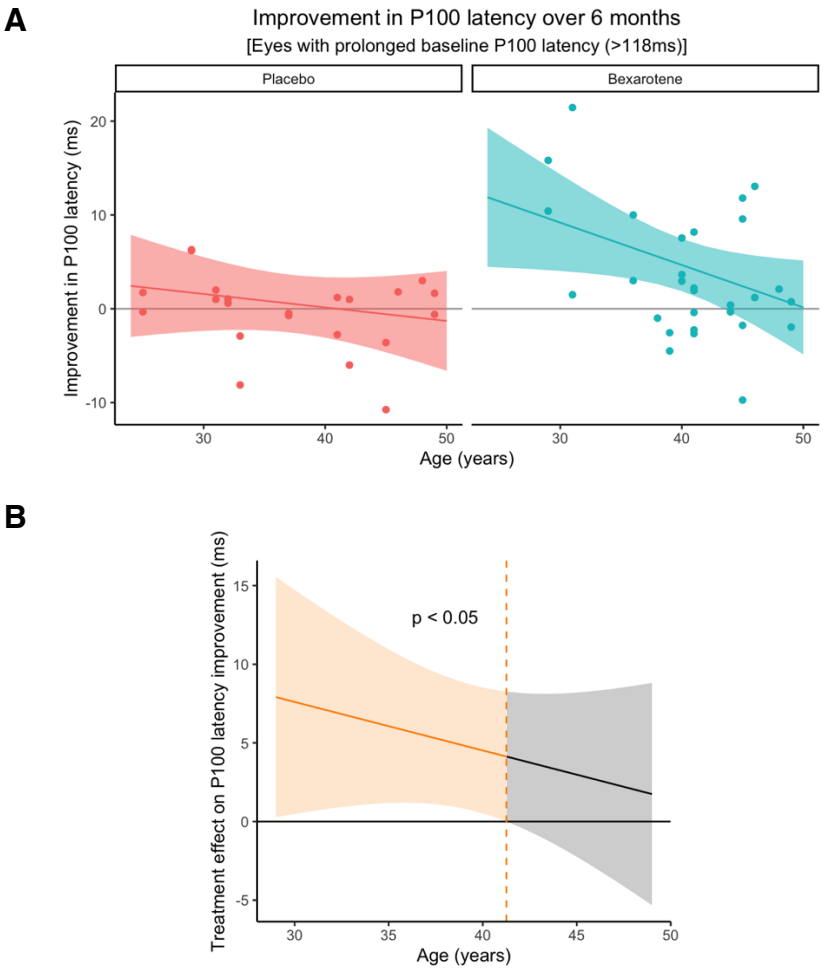


Figure 3.1. A: Variation of P100 latency improvement (reduction) with patient age for eyes with a prolonged baseline latency (>118 ms). Each datapoint represents an eye, and the 95% confidence interval for the model is shown. B: Treatment effect (bexarotene versus placebo) from (A) as a function of age.

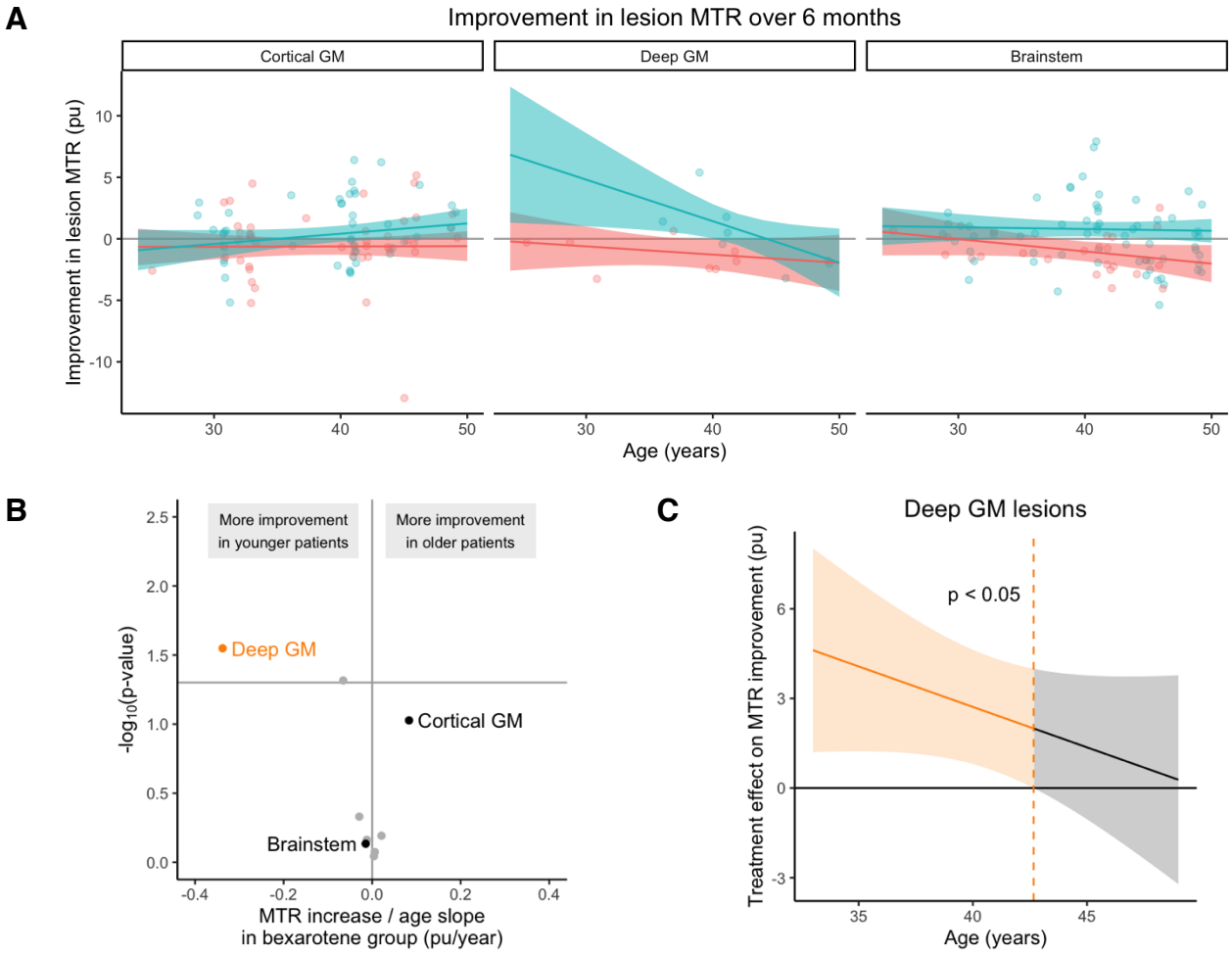


Figure 3.2. A: Variation of MTR improvement (increase) with patient age for the three regions with greatest remyelination. The bexarotene group is shown in blue and placebo in red. Each datapoint represents a lesion, with superimposed horizontal jitter to better visualise lesions with the same patient age. The 95% confidence interval for the model is shown. B: Volcano plot showing the effect of age on lesion MTR improvement within the bexarotene group for the locations plotted in (A). Other brain regions are shown in grey for comparison. C: Treatment effect (bexarotene versus placebo) for deep grey matter lesions as a function of age. GM = grey matter, MTR = magnetization transfer ratio.

Discussion

Here, I demonstrate – for the first time in humans – that the response to a remyelination-promoting drug decreases in older patients. Bexarotene, an RXR agonist, significantly improved VEP latency and deep grey matter MTR only in patients up to their early 40s. This is consistent with the comprehensive evidence-base describing remyelination failure with age in rodent models,[100,414,415] and clinical studies showing age-dependent accumulation of disability amongst people with MS.[256,416,417] Indeed, the fifth decade of life is typically the age at which patients develop progressive disease, regardless of prior disease course,[419] giving a possible indication of the timescale of age-related remyelination decline in humans.

I observed heterogeneity in age-dependency amongst the brain regions that were most responsive to bexarotene: remyelination declined with age in the deep grey matter, but this effect was not seen in cortical grey matter or brainstem lesions. Deep grey matter volume loss can be seen very early in the course of MS,[420] and histological assessment found premature accumulation of iron deposits and oxidised DNA in this region compared to white matter or cortex,[421] suggesting that deep grey matter may be particularly sensitive to the effects of age. In contrast, we found cortical grey matter remyelination to be more resilient in older patients, mirroring pathological findings that these lesions can actively remyelinate in the brains of patients into their seventies.[270]

This study is a post-hoc analysis of a trial that was not originally designed to assess the effect of age; as such, one limitation is the age range of participants, covering only young adulthood to middle-age (25-50). A more comprehensive picture of human remyelination and age will need recruitment of patients across the human lifespan. Additionally, our lesion numbers were relatively few in the deep grey matter (n = 16), and this finding will need to be replicated in a larger cohort. Nonetheless, this study advances the existing literature by measuring remyelination longitudinally in patients of different ages, using both structural and functional approaches.

This analysis contrasts somewhat with the RENEW trial, in which the best VEP latency response to opicinumab was seen in patients aged 33 and older.[422] Differences in the two study populations may underlie this disparity: the participants in CCMR One were older on average and with an established diagnosis of MS, whereas those in RENEW presented with a first episode of optic neuritis and no previous MS diagnosis. Additionally, we chose to focus on chronic optic neuropathy rather than acute optic neuritis. The intention of this was to avoid any confounding effects of active inflammation on the VEP; indeed, the age-related attenuation in VEP response in our study was strengthened when eyes with recent optic neuritis were excluded. One similarity between the studies was the poor response of older patients receiving placebo, suggesting little baseline remyelination in this group.

Any approach to promoting remyelination in MS might be limited by the intrinsic capacity of the aged CNS to repair. Encouragingly, this capacity can be enhanced in rodent models through interventions that rejuvenate an older animal's biological age, such as exposure to a youthful systemic environment,[268] intermittent fasting[100] or drugs including niacin[423] and metformin.[100] With the demographic of patients with MS getting older,[424] such interventions are likely to play an important role in emerging strategies to promote remyelination and reduce disability.

Chapter 4: The durability of the remyelinating effect of bexarotene

A follow-up sub study of CCMR One

Abstract

Remyelination has emerged as a critical therapeutic target in multiple sclerosis that has the potential to restore function and protect demyelinated axons. Successful trials of putative remyelination-promoting drugs depend on valid, non-invasive, and reliable outcome measures that are sufficiently responsive over short-duration trials. At this point, neuroimaging has not delivered sensitive and specific measures of remyelination; instead, electrophysiology is proving invaluable in phase II remyelination trials.

The shortening of visual evoked potential (VEP) latency directly reflects remyelination in the visual pathway and has now shown positive treatment effects in clinical trials of clemastine, opicinumab and bexarotene. Meanwhile, the mounting recognition of the constraints of the full-field VEP has led to the increasing application of multifocal VEP, such as in studies of nanocrystalline gold, opicinumab and metformin. However, it is not known how soon after a bout of acute optic neuritis these measures can be reliably deployed. An additional unresolved question is centred on the durability of VEP latency reductions after trials of remyelination drugs – such as bexarotene.

Unfortunately, the studies centred on these research questions were halted by the COVID-19 pandemic not long after I was granted ethical approval. However, I secured authorisation to proceed to recall the Cambridge CCMR One participants, successfully submitting that collecting these trial data fell within level 2 research of the NIHR recovery strategy. In so doing, I was able to demonstrate persistent improvements in full-field VEP latency in follow up assessments of 20 (of 31)

Cambridge-based participants from the CCMR One trial. I additionally evaluated multifocal VEP in these participants, allowing cross sectional comparisons of these measures with the full-field technique.

Contribution statement

In March 2020, I travelled to Sydney to visit Professor Sasha Klistorner and developed the skillset to independently conduct and analyse the multifocal visual evoked potentials. I conceptualised and designed this research study. I was granted ethical approval and commenced patient recruitment and assessment. I performed all of the assessments and undertook the statistical analyses of these data.

Background

It has been extensively chronicled that remyelination of demyelinated axons in the spinal cord by newly formed oligodendrocytes results in recovery of nerve conduction velocities[203,425] and is able to restore normal neurological function.[426] The pressing need to capture and track remyelination in humans has led to a resurgence in the use of electrophysiology to document this biology.

Full-field visual evoked potentials (FF-VEPs) have been used since the 1970s to detect occult visual pathology: increased latency with preserved waveform morphology being considered a sign of demyelination.[427] Importantly, there is now direct evidence from feline models that shortening in VEP latencies corresponds directly with the degree of remyelination;[418] strengthening the position that reductions in VEP latency reflect remyelination rather than ion channel redistribution or plasticity.[428] This engenders more confidence in the results returned by trials including ReBUILD,[277] RENEW,[285] and CCMR One (Chapter 2). Of course, as described in my introductory chapter, FF-VEPs have to contend with the problems of phase cancellation, responses dominated by the inferior visual field, and potential insensitivity to small peripheral field abnormalities.[429] This prompted the testing of discrete portions of the field through the technique of multifocal VEPs (MF-VEPs),[353,430] which seem more sensitive in the setting of remyelination trials.[366]

In the course of my PhD, I recognised 2 important questions. First, that if VEPs are to be deployed as outcome measures, it is essential to understand the time course and extent of electrophysiological recovery in eyes after acute optic neuritis (AON) and in eyes with subclinically delayed VEPs. A combination of prospective[357] and retrospective[358] analyses of FF-VEP suggest that exclusion of patients within 2 years of the last clinical episode of AON is appropriate; the same stability has not yet been clearly demonstrated for MF-VEP.[360] Second, it is unclear if the latency improvements observed following exposure to putative remyelinating drugs are durable. In ReBUILD, there was a signal that this is the case: latency reductions were still evident 2 months after clemastine cessation in one of the treatment

groups.[277] However, in CCMR One (Chapter 2), the final FF-VEP was completed on the day of treatment discontinuation.

Methods

I designed a research project entitled studying progressive remyelination in nerves by grading electrophysiological recovery (SPRINGER), with the intention of (a) quantifying how MF-VEP measures change over time in people living with multiple sclerosis, including after a recent episode of acute optic neuritis and (b) determining if latency changes seen in previous remyelination trials are durable, and potentially more significant, after the conclusion of the study. As part of the preparation for this, I wrote the study protocol and related documentation before navigating the ethical and local trials unit approval process; this study was authorised by the Wales REC 7 committee (20/WA/0294). Unfortunately, due to the constraints of the pandemic, which closed the Cambridge clinical research centre to observational studies from March 2020, I have only been able to address the latter research question

Subjects

Participants were recruited with a diagnosis of multiple sclerosis who had previously been part of the CCMR One trial based in Cambridge (Chapter 2). All had a diagnosis of RRMS and had been treated with dimethyl fumarate during their trial participation. Data were collected on their relapse history, optic neuritis history, and any changes to disease modifying treatments. Meanwhile, healthy subjects were recruited as controls for the VEP assessments. All participants gave written informed consent.

Equipment and protocols

Both full-field and multifocal VEPs were assessed using a Vision-Search Plus system (VisionSearch, Sydney, NSW, Australia); the FF-VEPs undertaken during the CCMR One trial had been performed on a Synergy system (Optima Medical Ltd, UK). In both instances FF-VEPs were elicited by a 2 Hz reversing check pattern of size of 60-min of arc with signal recorded from a channel formed between gold-cup

electrodes positioned frontally in the midline and 2.5 cm above the inion (Fz-Oz). Between 3 and 5 averaged recordings were taken per eye, and the weighted average of these used to measure the N75, P100 and N145 latencies and the amplitude between the N75 and P100. Multifocal VEP testing instead recorded signal from four gold-cup electrodes (Grass Technologies): a vertical channel (using electrodes placed in the midline 2.5 cm above and 4.5 cm below the inion) and a horizontal channel (via electrodes placed 4 cm either side of the inion). VEP signals from each of the 56 segments were amplified (100,000 times) and band-pass filtered (1-20 Hz): amplitude was taken as the largest peak-trough signal from either the vertical or horizontal channel within an interval of 70-200 ms, latency was defined using the second peak of this maximum amplitude wave (Figure 4.1). Segments with no detectable amplitude (where the amplitude of the response was less than two times the noise level of the trace within the interval 400–1000 ms) were assigned an amplitude of 0 nV but not assigned a latency; the MF-VEP amplitudes and latencies were then averaged from each segment.

All participants had a further expanded disability status scale (EDSS) assessment. Additionally, all participants had their Sloan low-contrast (1.25% and 2.5%) visual acuity recorded for each eye at 2m distance with a PrecisionVision wall chart. Acuity was recorded as the logarithm of the minimum angle of resolution (logMAR).

Statistical analysis

Statistical analysis was performed using R statistical software. To evaluate the pattern of FF-VEP amplitude and latency change, I tested the treatment effect using a mixed effects model applied with random effects for participant and eyes within participant, adjusting for fixed effects for baseline VEP latency and minimisation variables: age (≤ 40 /> 40 years), gender, and EDSS (≤ 4.0/> 4.0 score). For the EDSS analysis I used multiple regression of the change in EDSS on a group indicator with the same covariates as above. Cross sectional comparisons of MF-VEP parameters and low contrast visual acuities were undertaken using either t-tests or Mann-Whitney U tests where any violation of distribution assumptions was evident. A p-value of ≤0.05 was considered statistically significant.

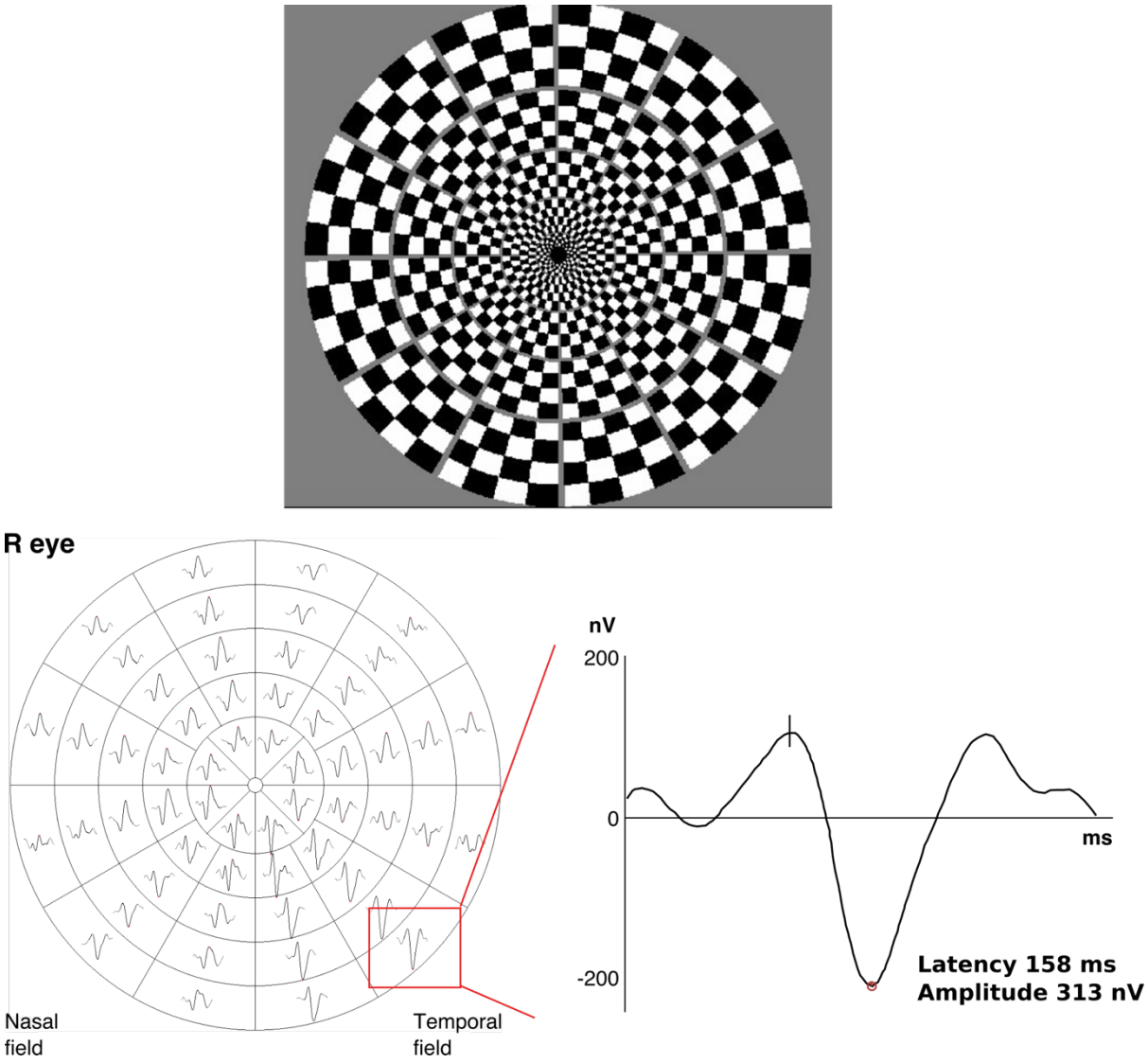


Figure 4.1. The multifocal visual evoked potential (MF-VEP). *Above:* cortically scaled stimuli used in MF-VEP reverse in pseudorandom sequence. *Below*: example of recording performed by myself on the right eye of a patient with chronic optic neuropathy, with an expanded trace from one segment showing delayed latency in the vertical channel. The average amplitude and latency for this eye was 213 nV and 155 ms respectively.

Results

Between December 10th, 2020 and April 6th, 2021, 21 out of the 31 CCMR One participants from Cambridge consented to participate; one was unable to attend on account of sickness resulting in a sample of 20 people with MS (12 were from the bexarotene arm and 8 from the placebo arm of the trial, Table 4.1). Clinical relapses in two participants, radiological activity in one, and lymphopaenia in one further participant, had led to treatment escalation from dimethyl fumarate. Two participants had since been diagnosed with SPMS; both remained on dimethyl fumarate at the time of enrolment. No participants had had an episode of acute optic neuritis since their participation in CCMR One.

	Bexarotene	Placebo	Healthy Control
Total number of participants	12	8	10
Number converted to SPMS	1	1	NA
Age, years; mean (SD)	44.3 (6.3)	42.8 (4.8)	32.8 (14.9)
Sex Female Male	 7 5	 3 5	 6 4
Disease duration, years; mean (SD)	11 (5.9)	8.4 (5.8)	NA
Number of patients with clinical relapses since CCMR One	1	1	NA
Disease modifying drug Dimethyl Fumarate Cladribine Fingolimod	 9 2 1	 7 1 0	NA
Total number of VEP recordings with sufficient quality for inclusion (number of eyes)	24	15*	20
Baseline P100 latency, ms; mean (SD)	132.3 (17.7)	126.1 (22.3)	NA

Table 4.1. Baseline variables of those who attended the CCMR One follow-up visit. Data are presented by trial group, alongside the information for the healthy controls who additionally undertook visual evoked potential (VEP) assessments. SPMS: secondary progressive multiple sclerosis. *one eye included in this group had an unrecordable P100 latency at the baseline visit of CCMR One.

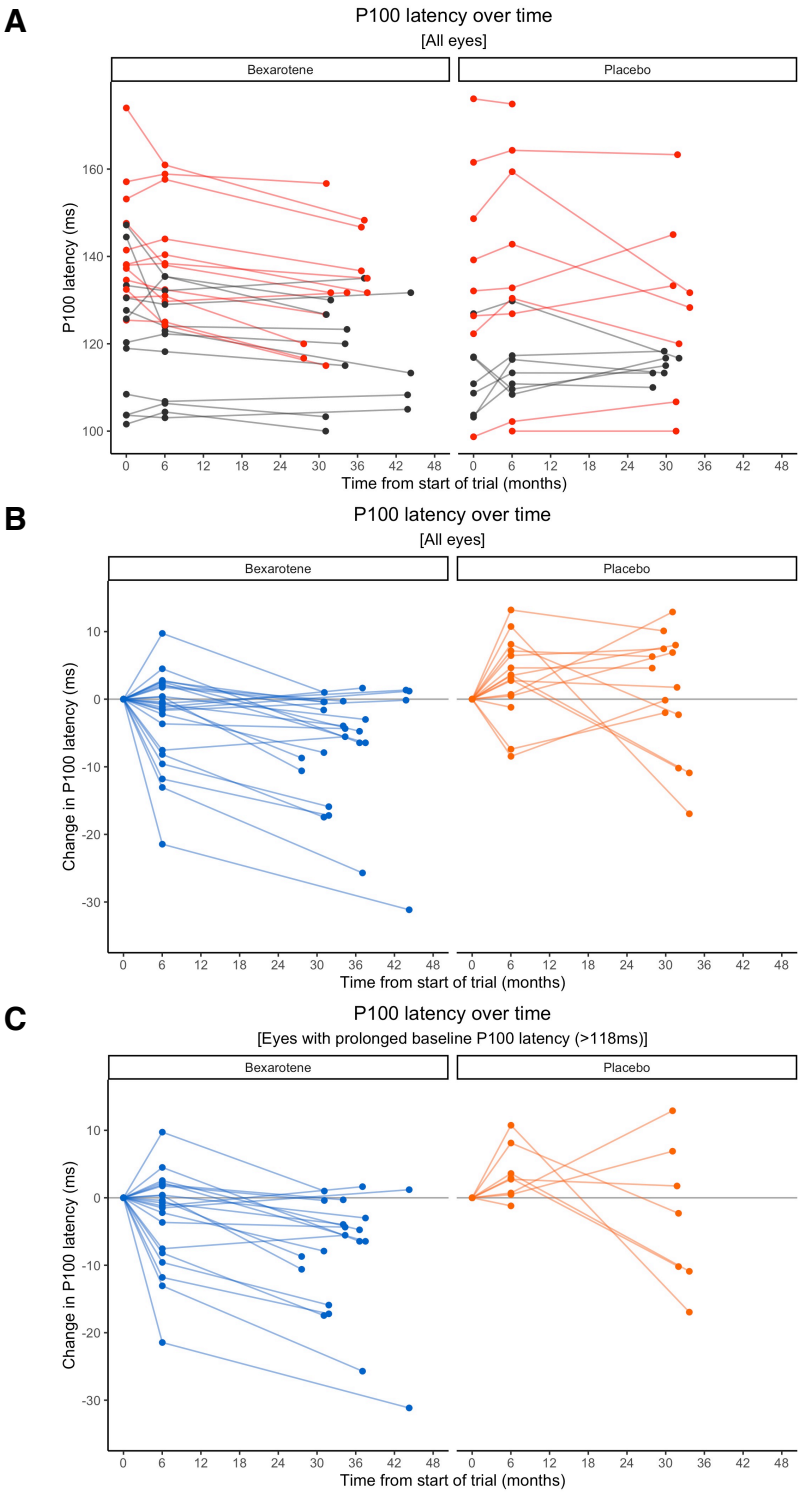


Figure 4.2. The change in full-field P100 latency over time. A: the progression of the P100 latency for each eye over the course of the baseline and 6-month visits of CCMR One, and the follow up assessment, divided by treatment group. Red lines indicate eyes previously affected by a clinical episode of acute optic neuritis. B and C: the change in P100 latency for all eyes and for just those eyes with a baseline P100 latency >118 ms, respectively, over the 3 VEP assessments.

38 out of 40 full-field VEP recordings (95%) were of sufficient quality to be analysed at both the CCMR One baseline and the follow up visit recordings. With all eyes included (24 bexarotene and 14 placebo) there was a statistically significant difference between the follow-up and baseline P100 latencies of the two trial arms: the adjusted treatment difference was -7.79 (95% CI -14.76, -0.82) ms, p=0.044 (Figure 4.2). However, when only eyes with a baseline P100 latency >118 ms were included (20 bexarotene and 7 placebo), the trend to improvement in P100 VEP latency remained but was not statistically significant: the adjusted treatment difference was -5.39 (95% CI -16.11, 5.32) ms, p=0.343.

A similar pattern was observed for the adjusted change in N145 latency between the follow-up and baseline trial visits, which was -5.95 (95% CI -10.80, -1.10) ms (p=0.021) with all eyes included (23 bexarotene and 14 placebo), and -4.02 (95% CI -11.48, 3.43) ms (p=0.319) when the analysis was restricted those with a baseline P100 latency >118 ms (19 bexarotene and 7 placebo). Meanwhile, there were no differences in the adjusted changes of the N75 latency: for all eyes this was 1.85 (95% CI -9.66, 13.37) ms (p=0.756) and for those eyes with delayed baseline P100 latency this was 2.95 (95% CI -17.25, 23.15) ms (p=0.800).

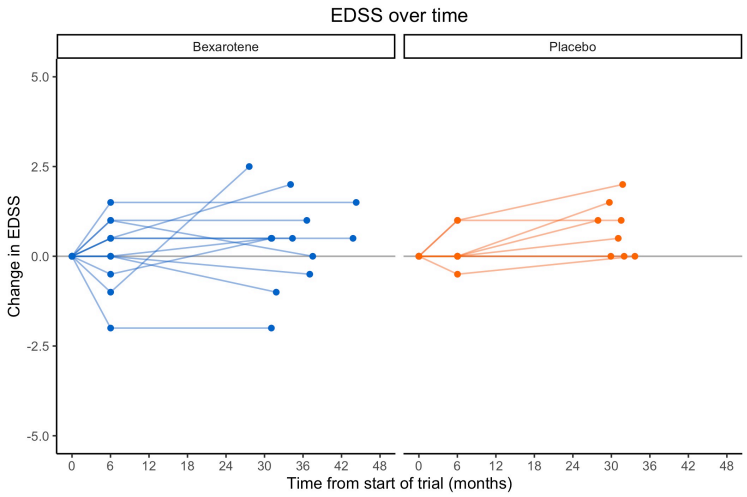


Figure 4.3. The change in EDSS. The change in the EDSS over the course of the baseline and 6-month visits of CCMR One, and the follow up assessment, subdivided by treatment group.

3 participants – all from the bexarotene group – had had an improvement in disability between the CCMR One trial baseline and the follow-up visit (Figure 4.3). However, there was no treatment difference between the two groups: the EDSS difference, adjusted for age and gender, was -0.31 (95% CI -1.37, 0.74), p=0.569.

I also performed multifocal VEP (MF-VEP) assessments on each of the 20 participants at the follow-up visit, in addition to 10 healthy controls. There were statistically significant differences between the mean latencies of both the bexarotene (152.5 ms) and placebo (157.3 ms) groups, with the healthy controls (137 ms; $p<0.001$ in each instance, Figure 4.4A). There was, however, no difference between the mean MF-VEP latencies of the two CCMR One groups (p=0.413). MF-VEP amplitudes did not differ between the 3 groups (Figure 4.4B). Linear regression analysis demonstrated a good correlation between the P100 latency of the full-field VEP and the averaged latency of the MF-VEP of those participants with MS: coefficient of correlation (r) was 0.81 ($p<0.0001$) (Figure 4.5A). The equivalent correlation of amplitudes between the two tests was less strong (Figure 4.5B).

I additionally examined 2.5% Sloan low contrast letter acuity, and a statistically lower LogMAR was evident in the bexarotene-treated group (0.51), than those who had been treated with placebo (0.69), p=0.021, corresponding to an improvement of 9 letters on the chart. There was, however, no between-group difference at the 1.25% contrast level (Figure 4.6).

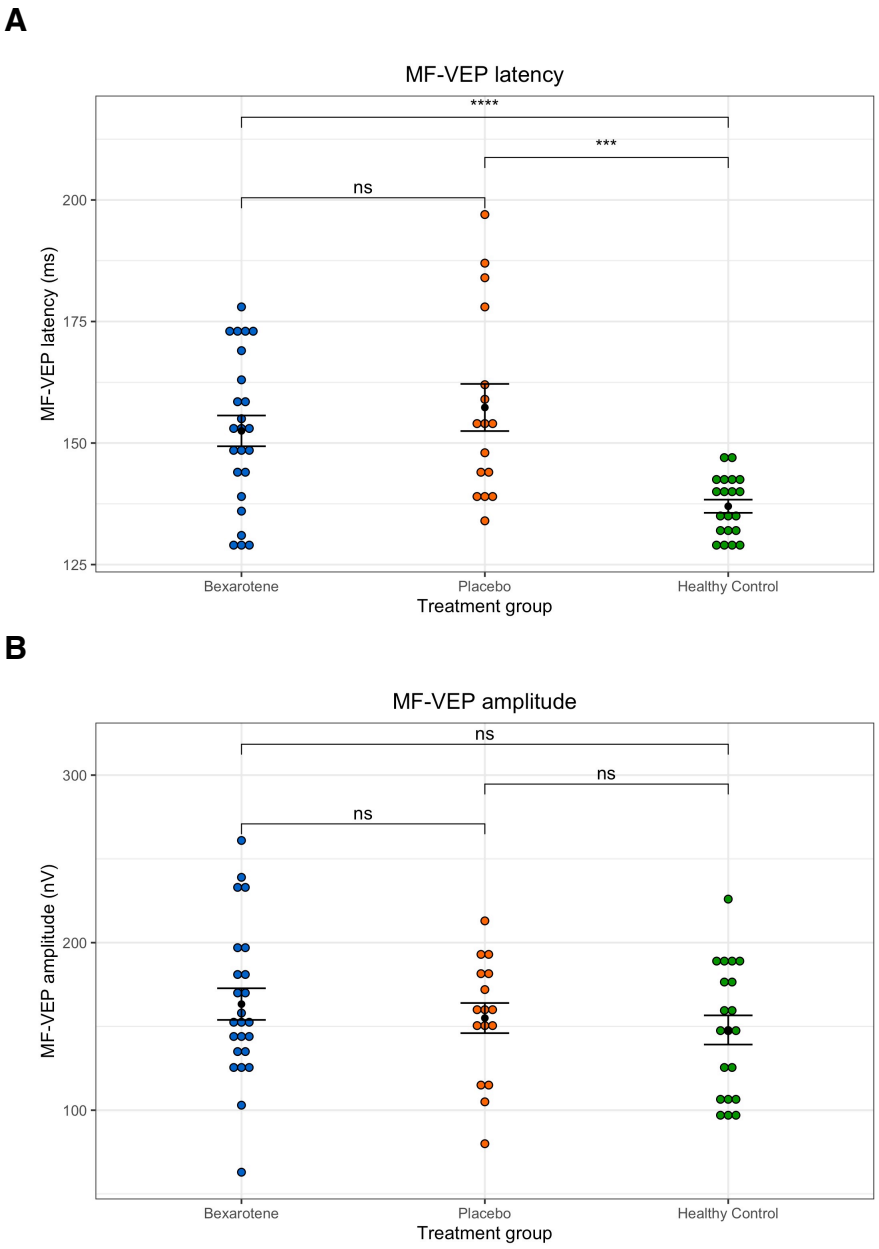
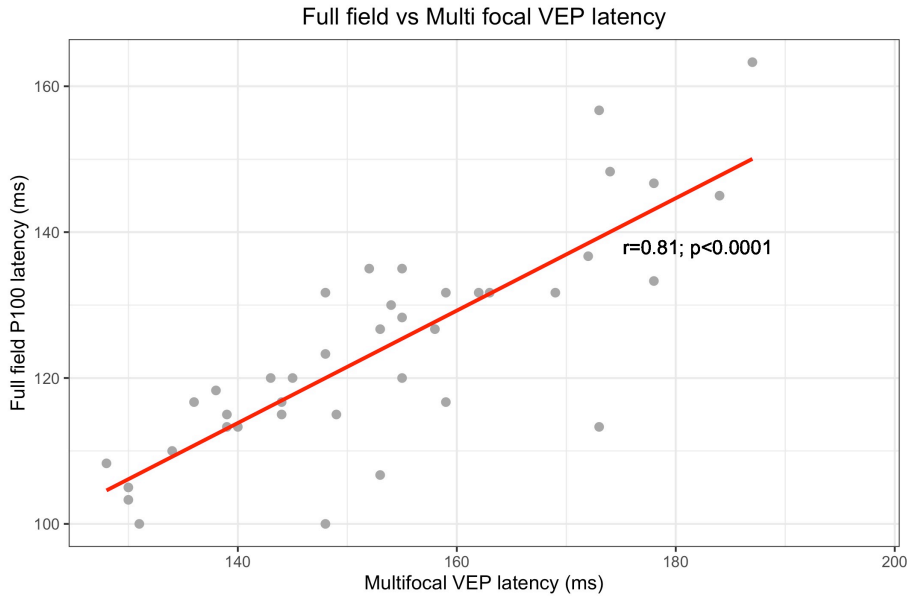


Figure 4.4. Between group differences in multifocal VEP latency and amplitude. Plotted here is the MF-VEP latency (A) and amplitude (B) for each eye, performed at the follow-up visit, subdivided by CCMR One treatment group, or healthy control participants. Bars are standard errors around the group mean. *** p<0.01, **** p<0.001.

A



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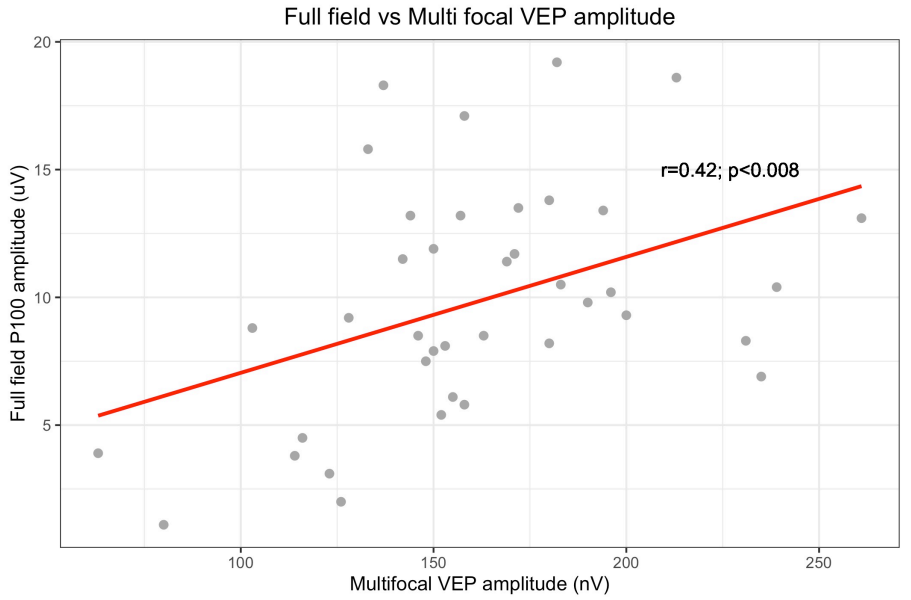

Figure 4.5. Correlation between latencies (A) and amplitudes (B) of full-field and multifocal VEP. Values refer to the P100 component of the full-field VEP and averaged latency and amplitude of the multifocal VEP.

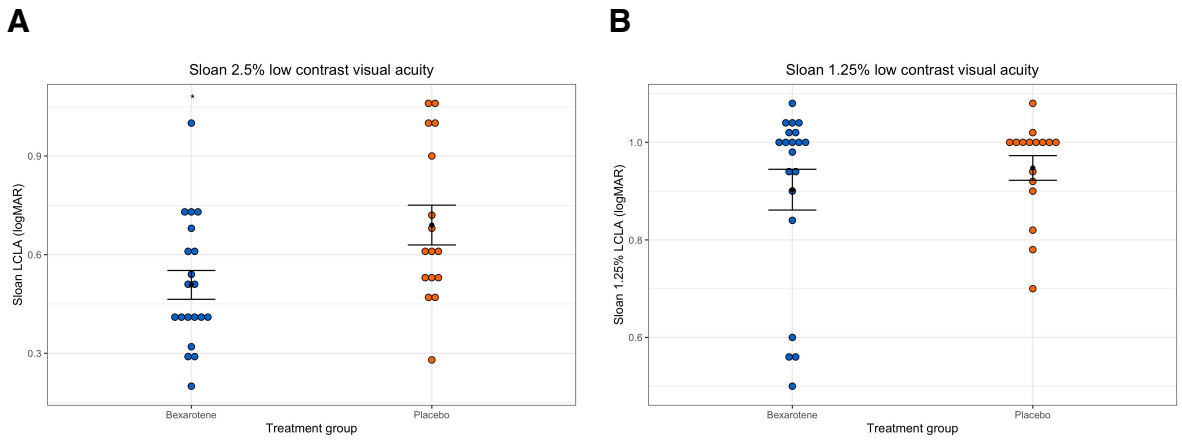

Figure 4.6. Between group differences in low contrast visual acuity. Shown here is the logarithm of the minimum angle of resolution (logMAR) using a Sloan 2.5% and 1.25% low contrast wall chart, performed at the follow-up visit, subdivided by CCMR One treatment group. Bars are standard errors around the group mean.

Discussion

This follow-up study to CCMR One has shown that the full-field VEP latency improvements observed in the original trial are durable, with a larger treatment effect seen in the all eye analysis, which met the level of statistical significance despite a sample size less than half of that seen in CCMR One itself. That the change in those eyes with a baseline P100 latency of >118 ms was not statistically significant, despite a larger treatment effect size relative to the CCMR One trial, might be attributable to the very small numbers in the placebo group (just 7 eyes in this analysis) as well as the spread of their latencies. A further finding was that, between the baseline and the follow up visit, the 3 participants who displayed an improvement in disability were all in the bexarotene group from the trial; a point that should be interpreted with caution given there was, again, no treatment difference for EDSS between the two groups when all participants were included. Certainly, while these data support the finding that bexarotene has a biological effect in humans and additionally suggest that this might have a long-term benefit years after the treatment period has concluded, they should be interpreted in light of the adverse events we

observed that we feel precludes the use of bexarotene in the treatment of people with MS (Chapter 2).

Durability of VEP latency changes after exposure to a putative remyelination drug has been shown before. In the ReBUILD study of clemastine, sustained VEP improvements were observed 2 months after clemastine discontinuation in one of the trial groups.[277] Additionally, in the RENEW trial of opicinumab, the VEP was repeated 8 weeks after IMP discontinuation, at which point the treatment difference in the per-protocol sample had increased to -9.1 ms, from -7.6 ms at the end of the 24 week treatment period, and then met the test of statistical significance.[285] This sub study of CCMR One is the first conducted at a time years remote from participation in a remyelination trial. It additionally begins to address a potential criticism of CCMR One; that the end of trial VEP assessment occurred while the patients were still taking bexarotene (and, indeed, levothyroxine), which might potentially have affected VEP latency through mechanisms outside of structural changes in myelination.[405]

There are, however, limitations to these findings. First is the relatively small number of participants, taken from only one of the trial sites, which introduces the possibility of selection bias. Second, a potential confounding factor is that the follow-up clinic used a VS+ device, in comparison to a synergy (Optima medical) set-up that had been used during the trial. Third, 2 participants in the bexarotene group had had their DMT treatment escalated to cladribine in the period between the end of trial participation and follow-up; this could be interpreted as being contributory to VEP improvements (through more effective control of inflammation), though the converse – that disease activity despite dimethyl fumarate was greater in these patients, thus the treatment escalation – could also be argued.

The optimal biomarker to precisely capture and track demyelination, remyelination and neuroaxonal injury is not known. No imaging measure of remyelination has yet satisfied the 5 conditions for use in neuroprotective and reparative MS trials set forth by Barkhof et al.[431]: pathological specificity, reproducibility, sensitivity to change,

clinical relevance, and responsiveness to treatment. The latency of the VEP accurately reflects pathological quantification of myelin in animal models of optic neuritis,[432] is sensitive to remyelination,[418] correlates with disability in people with MS,[350] and has now been shown to be responsive to treatment across 3 trials: CCMR One (Chapter 2), ReBUILD,[277] and RENEW.[285,366] The main limitation to the VEP in this context is the stringent requirements it places on participant selection criteria on account of the need for demyelination of the visual pathway and the high test-retest variability seen in the setting of acute optic neuritis.[358] MF-VEPs are particularly appealing as they are more reproducible than FF-VEP,[433] and are less subject to the ceiling effect (disappearance of the waveform) that can limit the usefulness of FF-VEP in patients with advanced disease.[388]

In performing cross sectional analyses of the MF-VEP latency in these participants there was no significant difference between the bexarotene and placebo groups at the follow up visit; both were prolonged relative to the group of healthy volunteers. In line with previous work,[434] there was a significant correlation between the FF-VEP and MF-VEP latencies at the follow-up visit ($r=0.81$, $p<0.0001$). This was less clear when it came to the equivalent comparisons of amplitude ($r=0.42$, $p=0.008$). A possible explanation is that the technique for quantification of amplitude is very different between the two tests; while the N75-P100 wave is used in full-field VEP, in MF-VEP the waveforms generated are of significantly different morphology. Another contributing factor is that the origins of the recorded signal in each instance is different: FF-VEPs mostly originate from a few central degrees of the visual field, while averaged MF-VEPs have a significant contribution from peripheral field.[359,360] It is therefore easy to envisage a situation of a patient with a peripheral defect having a normal FF-VEP but an abnormal MF-VEP. The superior correlation seen for latency might be explained by the smaller inter-subject variability when compared to amplitude.[434] In any case, the results of this study highlight the potential utility for MF-VEP testing in remyelination trials which, as described in the introduction, may allow for higher spatial resolution of remyelination of the visual pathway.

Chapter 5: The Cambridge Centre for Myelin Repair trial number Two (CCMR Two)

A phase IIa, randomised, double-blind, placebo-controlled clinical trial of the ability of the combination of metformin and clemastine to promote remyelination in people with relapsing-remitting multiple sclerosis already on disease-modifying therapy

Abstract

Promotion of endogenous remyelination represents the most realistic prospect for a neuroprotective treatment in MS. Clinical trials have deployed drugs, such as bexarotene and clemastine, to target the rate limiting step in this process: differentiation of oligodendrocyte progenitor cells (OPCs). Now, preclinical research has shown that metformin can reverse an age-associated deficit in the responsiveness of OPCs to pro-differentiation factors. The purpose of the Cambridge Centre for Myelin Repair trial number Two (CCMR Two) is to evaluate the efficacy of the combination of metformin and clemastine to promote remyelination in people with MS.

Patients with relapsing remitting MS (RRMS) will be randomised 1:1 to the combination of metformin and clemastine or matched placebos and followed for 24 weeks of treatment. All participants must be stable on a disease modifying therapy and have evidence of chronic stable optic neuropathy in at least one eye (defined by a latency of the visual evoked potential (VEP) ≥118 ms, and the absence of acute optic neuritis in the preceding 2 years). The primary outcome measure will be the change in the P100 latency of the full-field VEP between baseline and week 26. Secondary outcome measures will examine the change in multifocal VEP latency,

and the change in lesional MTR for lesions stratified by location and the cohort baseline median lesional MTR. With a sample size of 25 participants per arm, this will afford 80% power to detect a 3 ms reduction in VEP P100 latency.

In this chapter I set out the trial design, the rationale for participant and outcome measure selection, and all pre-specified analyses. In so doing, I expect to be able to detect the structural and functional consequences of remyelination within a sample size feasible for this single centre study. Following delays imposed by the COVID-19 pandemic, the trial will commence patient enrolment in 2021.

Contribution statement

Over the course of my PhD, I have led the design of this trial, put together a successful funding application to the MS Society, and submitted this for ethical approval. As described in Chapter 4, I formed a collaboration with Professor Sasha Klistorner at Sydney University to learn to conduct and analyse multifocal visual evoked potentials. I have additionally been part of a successful grant application to build our own vision research laboratory in Cambridge, which allows our research group to independently perform evoked potentials, visual fields, colour vision, low-contrast visual acuity, and saccadometry. I have written a protocol manuscript, which I intend to submit for publication once the trial has begun recruitment.

Background

Treatments capable of enhancing endogenous remyelination are needed to protect vulnerable axons from degeneration.[29] This can be achieved by promoting the differentiation of oligodendrocyte progenitor cells (OPCs) into mature, myelinating, oligodendrocytes.[400] Bexarotene, as described in Chapter 2, and clemastine, as described in Chapter 1, have both been shown to promote remyelination in animals and in phase II trials of people with MS by targeting this rate-limiting step.[246,277,291] However, it has previously been shown that this response becomes inefficient with age (Chapter 3 and ref[98]).

The scientific rationale for this trial has roots in the discovery that intrinsic changes take place within OPCs as they age that sees them become less responsive to the factors that normally enhance differentiation and remyelination.[100] Ribonucleic acid (RNA) sequencing from young and aged OPCs highlighted a significant contribution from the mTOR nutrient signalling pathway. Therapeutic modulation of this was achieved by subjecting 12-month-old rats to a six-month regime of alternate day fasting – these aged animals were subsequently able to remyelinate ethidium bromide-induced demyelinated lesions as effectively as younger animals. This effect was phenocopied by deploying the AMP-activated protein kinase (AMPK) agonist metformin; in aged rats, 3 months of treatment with metformin 300 mg/kg/day enhanced remyelination (Figure 5.1).[100]

In vitro differentiation assays on aged OPCs showed that metformin's effect is mediated through increasing their responsiveness to pro-differentiation factors (examples include T3, 9-cis-retinoic acid, miconazole and anti-muscarinic agents such as benzatropine, Figure 5.1).[100] Given that clemastine, which was identified to be pro-remyelinating in the same drug screens as benzatropine,[246,275] has a superior blood-brain barrier penetrance compared to other anti-muscarinic compounds, we have elected to test the combination of metformin and clemastine in this trial.

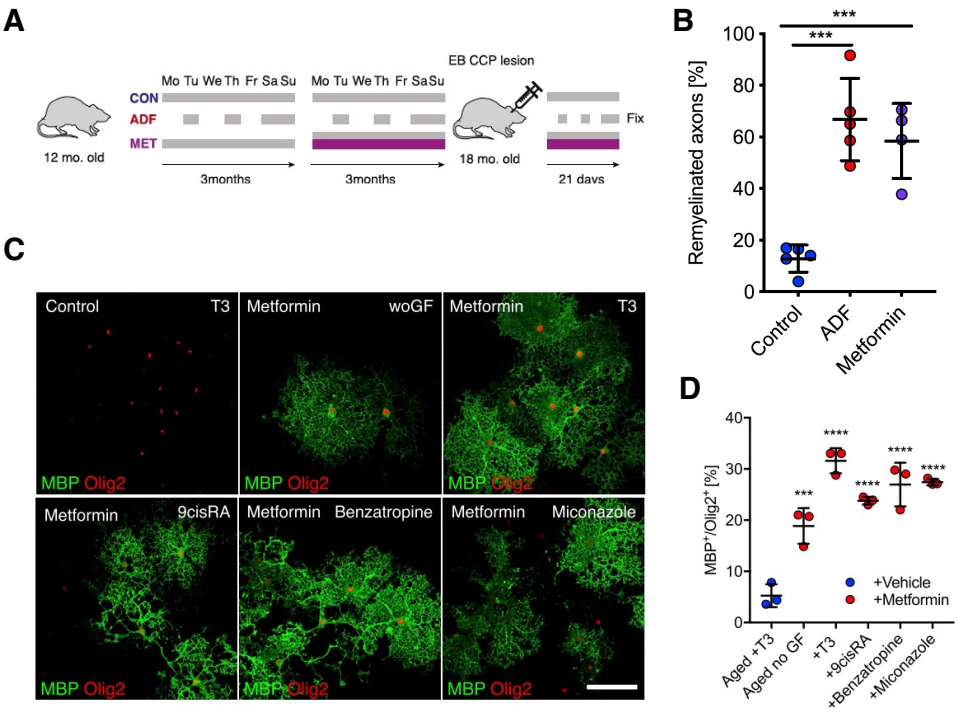

Figure 5.1. The scientific rationale for CCMR Two (reproduced from Neumann et al.)[100] A: schematic of *in vivo* experiment. 12-month-old female rats were divided into three groups: control, alternate daily fasting (ADF) and metformin (MET); metformin animals had ad libitum access to food and additionally received metformin at dose of 300 mg/kg bodyweight in their drinking water from the age of 15 months. At 18 months of age, demyelinating lesions were induced. B: histological quantification of remyelination revealed increased proportions of remyelinated axons in metformin-treated animals. C: *in vitro* differentiation assays support the use of metformin in combination with pro-differentiation drugs. D: from aged OPCs, differentiated $MBP^+/Olig2^+$ oligodendrocytes were formed in greater quantities in response to metformin alone. The addition of growth factors, including agents with an anti-muscarinic action (benzatropine) enhanced differentiation further still.

In fact, an initial aim of my PhD was to develop a clinical trial with calorie restriction as the intervention. In preparatory work to establish the feasibility of such a trial, I collaborated with the MS Society's research network (a group of people living with, or affected by, MS, who are trained to work with researchers to strengthen the quality and relevance of research by drawing on their personal experience of MS) in holding a focus group to better understand how a diet involving intermittent fasting might be tolerated and applied in a trial setting for people with MS. This was a productive exercise, revealing broad enthusiasm for participating in such a study, and valuable suggestions for how one might design a trial to additionally generate a reliable control population and monitor compliance (Appendix 1).

A dietary-intervention trial was, however, put on hold after a unique funding opportunity allowed us to first test the remyelinating effect of metformin. Frequency therapeutics, a US-based clinical-stage biotechnology company, hold a licence to exploit the patent on the combination treatment of metformin and anti-muscarinic drugs. Their approach has been to use combinations of small molecule treatments to activate progenitor cells; their early therapeutic work has focussed on regenerative treatments for sensorineural hearing loss. They expressed a desire to support MS clinical research and so held several meetings with our group. They subsequently made a donation to the MS Society to support their direct funding of this trial.

The outcome of this process has been the development of a phase IIa, randomised, double-blind, placebo-controlled clinical trial that will evaluate the ability of the combination of metformin and clemastine to promote remyelination in people with MS: the Cambridge Centre for Myelin Repair trial number Two (CCMR Two). Lessons drawn from CCMR One have featured strongly in our trial design. In particular, this trial tests the ability of this repurposed combination of drugs to reduce the latency of the VEP and to improve the MTR characteristics of chronic lesions stratified by their location and baseline tissue integrity. As previously described, the exploratory nature of outcome measures in phase II remyelination trials has been a particular obstacle. These need to be valid, reliable, and sufficiently responsive over a short-duration trial. Given that VEP latency strongly correlates with

myelination,[354,355] is highly reproducible in individuals (in particular with larger check sizes),[304] and has shown statistically significant effects in three trials (CCMR One, ReBUILD, and RENEW),[277,285] we have chosen VEP latency as our primary outcome measure. However, the recognition of regional remyelination in demyelinated chronic lesions has additionally prompted us to select MRI analyses sensitive to these changes.

Methods and analyses

Trial objectives

The primary objective of CCMR Two is to test the ability of the combination of metformin and clemastine to promote remyelination in demyelinated lesions in people with relapsing remitting multiple sclerosis (RRMS). Secondary objectives are: (i) to assess the efficacy of the combination of metformin and clemastine to promote remyelination in those with evidence of demyelination in the visual pathway as measured by the multifocal VEP latency; (ii) to evaluate the ability of metformin and clemastine to improve the MTR characteristics of MS lesions, stratified by baseline lesion MTR and lesion location; and (iii) to survey the safety and tolerability of these drugs in people with RRMS.

Exploratory objectives are to: (i) assess the clinical efficacy of this therapeutic combination by examining changes in Sloan low-contrast visual acuity, visual fields, colour vision and EDSS; and (ii) to evaluate the effects of metformin and clemastine on putative measures of neuroprotection including: optical coherence tomography, saccadic latency, amplitude of the full-field and multifocal VEP, brain atrophy, and serum neurofilament light.

Trial design

CCMR Two is a phase IIa, single-centre, double-blind, randomised, placebo-controlled, parallel groups add-on trial that compares the combination of metformin

and clemastine (maximally tolerated dose of metformin SR, up to 1 gram twice a day, alongside 5.36 mg clemastine twice daily) against matched placebos for 24 weeks in people with RRMS and chronic stable optic neuropathy. The primary end point is the change in full-field VEP latency between baseline and 26 weeks. 50 participants will be randomised in a 1:1 ratio, with an interim review by an independent data monitoring committee after 20 patients have completed their assessments to determine whether a sample size re-estimation should be considered. Participants will have a total of seven study visits with a final evaluation at week 28, all at the department of clinical neurosciences of Cambridge University (Figure 5.2).

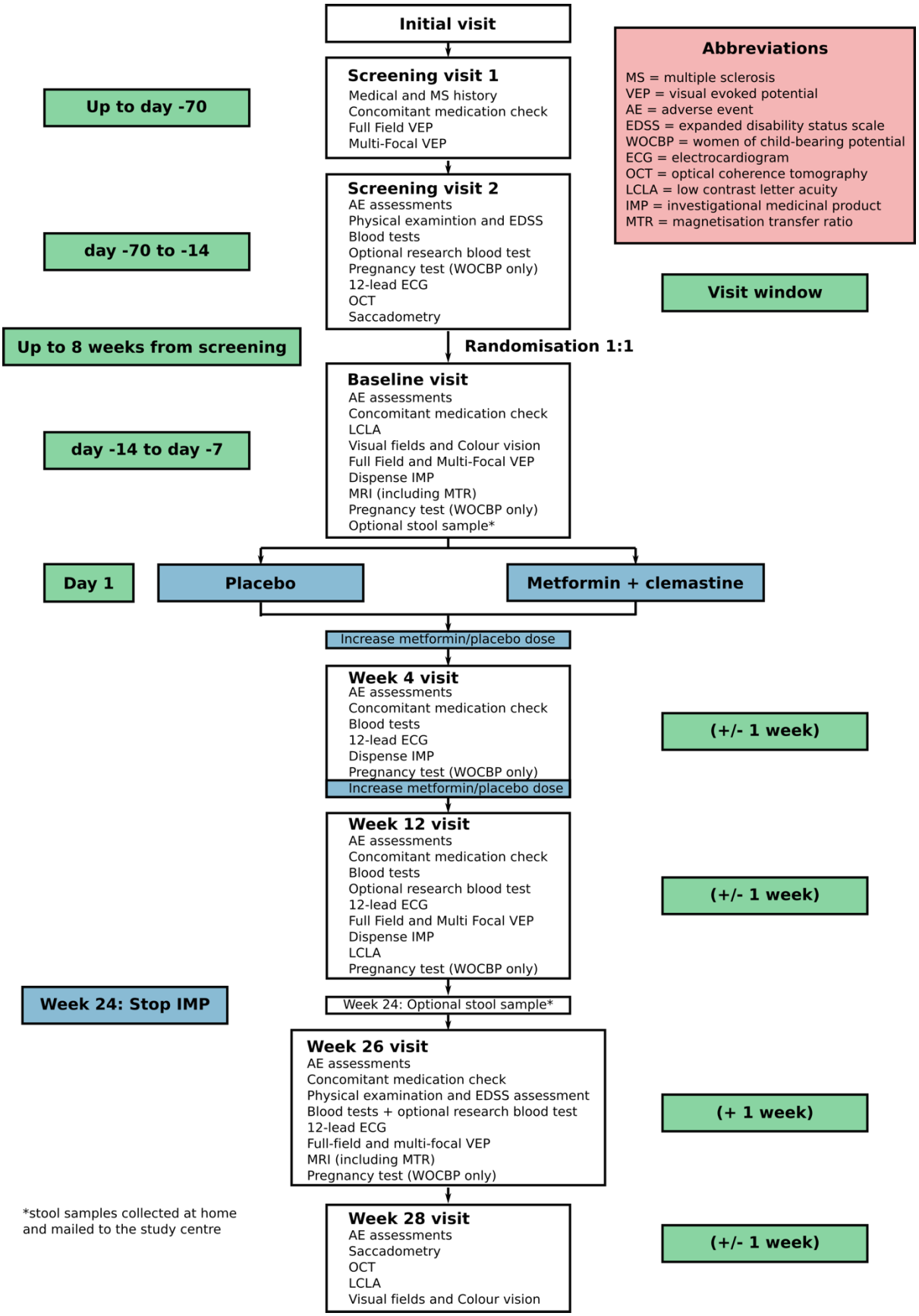

Figure 5.2. The Cambridge Centre for Myelin Repair trial number Two (CCMR Two) participant timeline.

Participant characteristics

Selection criteria for CCMR Two, alongside the rationale for each point, are shown in Table 5.1. Potential participants will be identified through specialist neurology clinics at the trial centre or referred to the trial team from other MS/neuroscience centres and hospitals. Candidate participants will then be seen in clinic or contacted via telephone to discuss the study directly with a member of the trial team and 'prescreen' to ensure that the interested participant is likely to fulfil the general criteria to enter the trial and be able to comply with the trial assessments and interventions. The potential participant will be given a copy of the participant information sheet (PIS) and informed consent form (ICF) and asked to contact the study team at an interval of no less than 24 hours to confirm if they would like to proceed to screening.

The screening visit will be divided into two sets of assessments. On the first, after signing of the informed consent form, the trial team will review the participant's medical history, imaging and records, to confirm the diagnosis of RRMS[115] and their eligibility for inclusion (Table 5.1). Participants will then have their screening VEP assessments to ensure there is detectable demyelination in the visual pathway. Data from CCMR One suggests that in the region of 36% of potential participants will not pass screening at this point; in the ReBUILD trial, it is noteworthy that 75 patients failed screening on this same criterion to yield 50 eligible participants.[277] If these assessments are satisfied, the individual would then be invited to return for a second series of screening investigations including blood tests, EDSS assessment, and optical coherence tomography (OCT) to ensure the criteria outlined in Table 5.1 are similarly met; a much lower attrition rate is anticipated at this point.

Inclusion criteria	Rationale
Age between 25 and 50 years (inclusive) at time of signing informed consent form*	Upper limit selected to reduce chance that age-related changes at MRI not misidentified as MS lesions. Lower limit to align cohort with the preclinical data showing remyelination failure is age-dependent
Relapsing-remitting multiple sclerosis as per the McDonald 2017 criteria, including an MRI brain satisfying the 2017 radiological criteria[115]	The expectation is that selecting RRMS patients will maximise the number of demyelinated MS lesions with intact axons,[28,401] thereby providing the substrate for remyelination
Full-field visual evoked potential (VEP) P100 latency in at least one eye of ≥118 ms	To ensure there is sufficient demyelination in the visual pathway to allow detection of remyelination. 118 ms is 2 SD greater than the mean of our control data, and the same threshold as that previously used in the ReBUILD trial[277]
Kurtzke EDSS step 0.0 - 6.0*	To maximise the number of intact axons; increased degrees of neurodegeneration would be anticipated at higher levels of baseline disability
At the time of screening, being treated with a stable dose for at least 6 months of a category 1 multiple sclerosis DMT or for at least 2 years with a category 2 DMT[11]*	This allows the regeneration of myelin to be studied in some degree of isolation from active inflammation
Exclusion criteria	**Rationale**
Female participants who are pregnant, lactating, planning pregnancy, or unwilling to use reliable contraception during the trial	Metformin not recommended in pregnancy and is detectable in breast milk. Clemastine should not be given in pregnancy or when breastfeeding
Retinal nerve fibre layer thickness on spectral-domain OCT <70 µm in the qualifying eye	Below this threshold there are severe decrements in visual function;[372] above this value implies there remain sufficient axons to remyelinate
A clinical episode of optic neuritis in the qualifying eye within the 2 years preceding screening	Following an attack of optic neuritis, VEP latencies are prolonged but a period of recovery follows for up to 2 years;[137,357] excluding these make improvements in latency more specific for drug-induced remyelination
Any concomitant use of oxybutynin, monoamine oxidase inhibitors (MAOIs), hypnotics or high-dose opiates at screening	These interact with clemastine. Oxybutynin additionally has potential remyelinating efficacy (though limited BBB penetrance)

Significant renal or liver impairment (eGFR <60 mL/min/1.73m^2; alanine aminotransferase > 3 times the upper limit of normal)	Metformin very rarely can cause liver dysfunction and the incidence of lactic acidosis rises with impaired renal function
People taking medication for Diabetes Mellitus at screening	Metformin would alter glycaemic control
People with a diagnosis of epilepsy	Clemastine contraindicated in epilepsy. VEP testing contraindicated in photosensitive epilepsy
Concurrent use of 4-aminopyridine or fampridine	These can improve VEP and saccadic latency[390,435]
History of prostatic hypertrophy, cardiac conduction block, stenosing peptic ulcer, pyloroduodenal ulceration, ophthalmologic disease including glaucoma, macular degeneration, and severe myopia (>-7 Diopters)	Clemastine contraindicated in these conditions. Severe ophthalmic disease due to causes outside of demyelinating optic neuropathy might impact on VEP and OCT assessments

Table 5.1. Selection criteria for the CCMR Two trial with corresponding justification. *age, disability, and disease modifying treatment category are additionally included alongside gender as stratification factors in the randomisation (main text) to ensure matching baseline characteristics between active and placebo groups. MS, multiple sclerosis; VEP, visual evoked potential; SD, standard deviation; BBB, blood-brain barrier; EDSS, expanded disability status scale; OCT, optical coherence tomography; eGFR, estimated glomerular filtration rate; DMT, disease modifying treatment.

Following documented permission from the potential participant when signing the informed consent form, their usual treating neurologist and/or GP will be contacted to detail their involvement in the trial; if they are not able to be randomised after attending screening, reasons for this will be communicated to both the patient and referring clinician. We will also maintain an anonymised log of all patients who are ineligible for the trial and all eligible patients that will not be randomised because they declined participation. This process will enable generalisation of the trial results in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines of 2010.[436]

Details of the interventions

Metformin

Metformin is a biguanide licensed for human use in the management of type 2 diabetes.[437] The primary target of metformin is the respiratory chain complex I.[438] Towards this, the drug induces mild and transient inhibition, increasing the cellular adenosine monophosphate (AMP) to adenosine triphosphate (ATP) ratio, leading to activation of AMP-activated protein kinase (AMPK), which regulates various target genes. It also functions to inhibit the mammalian target of rapamycin (mTOR), through both AMPK-mediated and AMPK-independent mechanisms. Ultimately this leads to reduced hepatic glucose production (inhibits gluconeogenesis and glycogenolysis), increased insulin sensitivity in muscle (improves uptake and use of glucose), delayed intestinal glucose absorption and reduced total cholesterol, low density lipoprotein (LDL), and triglyceride levels.[439]

Metformin, has previously been demonstrated to reduce inflammation in progressive and relapsing experimental autoimmune encephalomyelitis,[440] and has been used in an open label trial of 20 people with MS and demonstrated a reduction in the number of new or enlarging T2 lesions compared with placebo.[441] Now, the demonstration that it can also reverse an age-associated barrier to the ability of oligodendrocyte progenitor cells to respond to pro-differentiation factors and enhance remyelination,[100] provides a compelling rationale for its use in this trial.

Metformin has been used in clinical practice for over 60 years and its safety profile is well established. Metformin is contraindicated in those with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2 and the maximum dose is reduced in those with an eGFR between 30 and 60 mL/min/1.73m^2. As a result, the selection criteria applied to the trial will ensure only those with an eGFR ≥60 mL/min/1.73m^2 can be included. Helpfully, while metformin is an anti-diabetic agent, it never causes hypoglycaemia and requires no monitoring of blood glucose levels. Meanwhile, metformin is generally well-tolerated. Gastrointestinal (GI) upset occurs in up to 20%, leading to discontinuation in 5%, however this is often short lasting and can be

minimized by gradual up-titration of the dose.[442] Prolonged-release forms, offering a slower absorption rate, additionally improve tolerability.[443-445]

The oft-feared consequence of lactic acidosis with metformin use is usually predictable (in those with renal failure) and is a very rare event – between 3 and 9 events per 100,000 patient years only.[446] However, the summary of product characteristics for metformin highlight that it should be temporarily discontinued in circumstances that may precipitate renal injury (such as severe dehydration and vomiting) or with use of intravenous iodinated contrast.

Clemastine

Clemastine, is a first-generation (inverse agonist of histamine) anti-histamine that inhibits receptors of the H1 type. It has a number of licenced indications including allergic rhinitis, hay fever, allergic dermatoses, and urticaria. After preclinical work demonstrating the potential remyelinating effect of clemastine,[246] it was tested in a phase II clinical trial.[277] The ReBUILD study, discussed in detail in Chapter 1, showed a mean reduction in the VEP P100 full-field latency of 1.7 ms/eye ($p = 0.0048$) in its crossover model, among those assigned to 5.36 mg clemastine twice daily.

Clemastine is a safe medication with no serious adverse effects; in the aforementioned trial the drug was well tolerated, though was associated with fatigue. However, higher doses would be limited by its tendency to sedation, which seems likely to be problematic in a patient cohort in whom fatigue is a common complaint. The summary of product characteristics for clemastine suggests caution in those with narrow angle glaucoma, prostatic hypertrophy with urinary retention, a history of pyloroduodenal ulceration and cardiac conduction block; participants with these comorbidities will be excluded in the trial.

Drug supply, packaging and dispensing

In 2019, I worked with Cambridge University Hospital's clinical trials pharmacy and the University procurement team to navigate the tendering procedures required to

secure the delivery and manufacture of the trial drugs and matched placebos. RenaClinical provided the most competitive quote relative to the other responding vendors (GSTT pharmaceuticals, and Royal Free) and so were awarded the contract. Labelling will be blinded in line with the requirements of EU Good Manufacturing Practice Annex 13. Blinding will be maintained by coding of bottles. All will be packaged in polyethylene bottles containing the same number of tablets. Bottles will be dispensed according to the standard operative procedures of the clinical trials pharmacy of Cambridge University Hospitals NHS Foundation Trust. A log will be kept recording each dose of IMP dispensed to each trial participant.

Dosing regimen and modification

Following randomisation, at the baseline visit study drug will be dispensed from the pharmacy. This will include tablets of clemastine 1.34 mg (or matched placebos), and metformin SR 500mg (or matched placebos). Participants will be instructed to commence 5.36 mg twice daily of clemastine on day 1 of the trial and continue this for the 24-week treatment period. This dose has been selected as it is the same dose has previously been deployed in the ReBUILD clinical trial (with no treatment discontinuation in a cohort of 50 patients with RRMS), while the study also reports only 50% saturation of the target muscarinic receptor (and so we have not opted for a lower dose).[277] Participants will similarly be instructed to commence 500 mg twice daily of metformin SR on day 1. This will be up titrated to 500 mg mane, 1 gram nocte after 2 weeks, before a final dose increase to 1 gram twice daily at 4 weeks; this dose will continue for the remaining 20 weeks of the treatment period. This dose has been selected as, in the preclinical experiments, the rats consumed 250 mg/kg/day (Neumann, personal communication); this translates to approximately 2.5 grams per day for a human assuming a body surface area of 1.7 m^2.[447] With 2 grams/day being the maximum dose recommended of a sustained release preparation of metformin, which is better tolerated compared to the standard release formulation, we have elected not to increase this any further.

If adverse events occur that are attributable to metformin – most likely gastrointestinal side effects – the trial team may reduce the metformin dose. If

symptoms settle, then the dose may be increased again, at the discretion of the trial doctor. In the event of an unrelated illness, such as diarrhoea and vomiting requiring hospitalisation, then the metformin (or its placebo) should be temporarily discontinued.

Evaluation of adherence to study drug

Participants will be issued with a dosing diary at their baseline visit to record the number of tablets taken and to indicate any missed doses (and reasons for non-adherence). However, participants will also be asked to return any unused study drug and a pill count conducted. A cumulative total of 28 missed doses over the entire treatment period will trigger a review by the CI/PI for consideration of participant withdrawal.

Outcome measures

Visual evoked potentials will be performed at screening, baseline, week 12 and week 26; brain imaging will be performed at baseline and week 26; clinical efficacy will be measured by the EDSS at screening and week 26 and by Sloan visual acuity at baseline, week 12 and week 28 (Table 5.2).

Primary outcome: full-field VEP

The primary outcome measure of CCMR Two will be the change in the P100 latency of the full-field VEP between baseline and week 26 for each eye with a baseline latency of ≥118 ms. The latter timepoint, 2 weeks removed from the end of treatment (week 24), has been selected to ensure that more than five half-lives have passed prior to the final VEP assessment; in this way any change in latency cannot be attributed to a direct effect of the investigational medicinal product on conduction velocity (making any changes more specific to variation in myelination). An additional VEP will be performed at week 12 to facilitate exploratory analyses of the time course of remyelination; this period was sufficient to detect an effect in ReBUILD.[277]

Assessment	Screening 1	Screening 2	Baseline		Treatment phase				
Clinic visit number	1	2	3	No visit	4	5	No visit	6	7
Visit window	Day -70 to -14	Day -70 to -14	Day -14 to - 7	Day 1	Week 4 (+/- 7 days)	Week 12 (+/- 7 days)	Week 24	Week 26 (+ 7 days)	Week 28 (+/- 14 days)
Informed consent	X			Start IMP			Stop IMP		
Eligibility assessments	X	X							
Medical and MS history	X								
Physical examination[1]		X						X	
EDSS assessment		X						X	
Concomitant medication check	X		X		X	X		X	
Randomisation		X[2]							
Adverse event assessments		X	X		X	X		X	X
IMP dispensing			X		X	X			
IMP compliance check					X	X		X	
Safety bloods		X			X	X		X	
Optional research bloods (for storing serum and PBMCs)		X				X		X	
12 lead ECG		X			X	X		X	
Pregnancy test (women of childbearing potential only)		X	X		X	X		X	
Full-field VEP	X		X			X		X	
Multifocal VEP	X		X			X		X	
OCT		X							X
Visual acuity (Sloan 1.25%, 2.5% and 100%)			X			X			X
Visual fields			X						X
Cambridge colour test			X						X
Saccadometry		X							X
MRI imaging			X					X[3]	
Optional stool sample[4]			X				X		

Table 5.2. Schedule of assessments for CCMR Two. Study specific activity is shown for each visit, see text for additional information. [1]Physical examination to include: height (screening only), weight, pulse, and blood pressure. [2]Randomisation only to be undertaken once all screening assessments are complete and eligibility confirmed. [3]The second MRI can be undertaken on a separate day to the week 26 visit, if necessary, within a +7 day window. [4]Stool samples will be collected by participants at home, approximately corresponding to the baseline visit and week 24. Samples should be collected up to 1 week prior to these time-points. MS, multiple sclerosis; EDSS, expanded disability status scale; IMP, investigational medicinal product; PBMC, peripheral blood mononuclear cells; ECG, electrocardiogram; VEP, visual evoked potential; OCT, optical coherence tomography; MRI, magnetic resonance imaging.

VEPs will be assessed by means of a Vision-Search Plus system (VisionSearch, Sydney, NSW, Australia). The stimulus protocol has been selected to comply with guidelines from the International Society for Clinical Electrophysiology of Vision.[448] The visual stimulus will be generated on a high-resolution liquid crystal display, with a 2 Hz reversing check pattern of size of 60-min of arc. The participant will be seated 50 cm from the screen. All will be optimally refracted for near vision and pupil dilatation will not be required. Electrical signals will be recorded from a channel formed between gold-cup electrodes (Grass Technologies, West Warwick, RI, USA) positioned frontally in the midline and 2.5 cm above the inion (Fz-Oz); a ground electrode will be placed on the ear lobe and conductive gel (0.5ml) injected under each. Between 3 and 5 averaged recordings will be taken per eye, and the weighted average of these used to measure the N75, P100 and N145 latencies and the amplitude between the N75 and P100 (Figure 5.3). A blinded rater will be responsible for ensuring quality control of each VEP record, and for latency and amplitude determination.

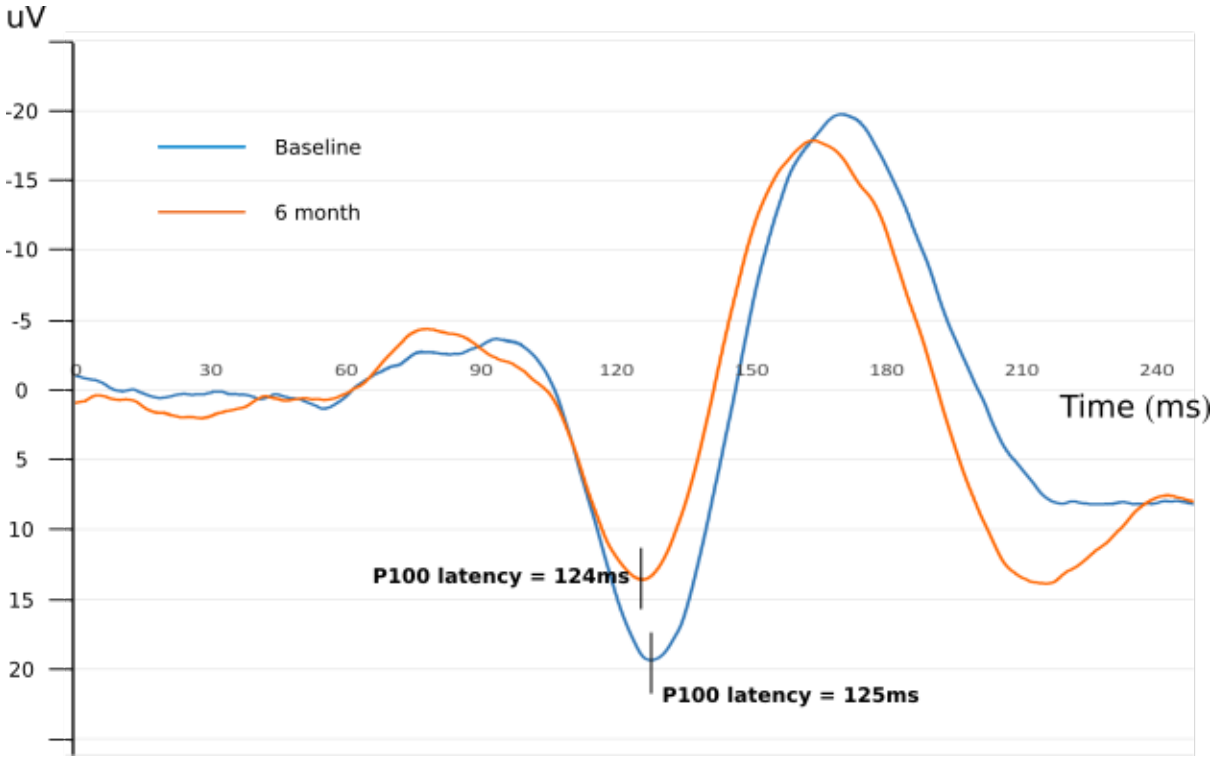

Figure 5.3. The full-field visual evoked potential. In response to a reversing 2 Hz 60 minute of arc checkerboard patterned stimulus, the averaged recordable signal takes the form of an N75-P100-N145 waveform. This shows 2 repeated measures from the same eye of an individual with MS, performed by myself, 6 months apart.

Secondary outcomes: electrophysiology

The first secondary outcome of the trial is to interrogate the change in multifocal VEP latency, between baseline and week 26, for those eyes with delayed latency at baseline. As detailed in the introductory chapter, multifocal VEPs overcome the problems of macular over-representation and phase cancellation,[353,359-361] allowing a more precise analysis of latency and amplitude abnormalities in people with optic neuropathy.[363-365] In this trial we will use the aforementioned equipment, this time to monocularly present a stimulus consisting of fifty-six cortically scaled segments, each containing a 4x4 checkerboard reversing in a pseudorandom sequence (Figure 4.1). Four gold-cup electrodes (Grass Technologies) will be used for bipolar recording: a vertical channel (using electrodes placed in the midline 2.5 cm above and 4.5 cm below the inion) and a horizontal channel (via electrodes placed 4 cm either side of the inion). The resultant VEP signals from each of the 56 segments are amplified 100,000 times and band-pass filtered (1-20 Hz). Amplitude is defined as the largest peak-trough signal from either the vertical or horizontal channel, within an interval of 70-200 ms, while latency is taken as the second peak of this maximum amplitude wave. Segments with no detectable amplitude are assigned an amplitude of 0 nV but are not assigned a latency. The software then averages the amplitudes from each segment and averages the latencies from only those segments with recordable signal. In order to be included in the analysis, the baseline recording must have recordable signal in at least 28 of the 56 segments, and a prolonged baseline latency is defined as being ≥151 ms. Progression analysis will then be performed across each of the 56 segments from each of the study visits by a blinded rater, to determine the change in multifocal VEP latency and amplitude for each affected eye.

Secondary outcomes: MRI

There are two secondary outcomes using MRI. First, is the change in lesional MTR, between baseline and week 26, for MS lesions stratified by location (e.g. cortical grey matter, brainstem etc.). Second is the change in lesional MTR, between baseline and week 26, for the lesions stratified by the cohort baseline median lesional MTR (especially those lesions with submedian MTR). MTR correlates with histopathological demonstration of myelin in MS lesions[319] and these analyses have

been selected to account for evidence presented in Chapter 2 that the degree of remyelination in an MS lesion is dependent both on its location, being greatest in the grey matter,[270,271,407] and the extent of its demyelination at baseline. We have prioritised chronic lesions in our analysis as remyelination trials centred on acute lesions require frequent MRI scans and large numbers of patients.[290]

In CCMR Two, MRI scans will be performed at baseline and at 26 weeks, using a Siemens 3T Prismafit scanner (Siemens, Erlangen, Germany) with 20-channel head-neck coils. Lesion identification, contouring and checking will be performed by blinded observers, with lesion location classified by using the brain parcellation from the volumetric T1 scan. Sequences sensitive to grey matter lesions will also be deployed including double inversion recovery (DIR) and phase sensitive inversion recovery (PSIR).[323]

Exploratory outcomes

CCMR-Two will additionally assess exploratory outcome measures to include the change in the Sloan low-contrast visual acuity (which will be measured as the logarithm of the minimum angle of resolution (LogMAR) for each corrected eye at 1.25% and 2.5% contrast levels), the change in colour vision (as measured by the Cambridge colour test), the change in Humphrey visual fields (as measured by the mean deviation in the Swedish Interactive Thresholding Algorithm (SITA)-Standard 24-2 protocol), and the change in disability (measured by the EDSS step).

The trial will also evaluate the effects of metformin and clemastine on putative measures of neuroprotection measured both before and after the treatment period: (i) optical coherence tomography (OCT), which visualises the proximal effects of demyelination that is often seen in the retrobulbar portion of the optic nerve;[367] (ii) saccadic latency parameters, which depend on a network of diffuse pathways and a lengthening in latency is likely to represent neurodegeneration;[391] (iii) the amplitude of the full-field and multifocal VEP; (iv) brain atrophy; and (vi) serum neurofilament light.

Safety and tolerability outcomes

Each study visit after the point of informed consent will capture data on adverse events (AEs), which will be evaluated by the investigator to establish its seriousness and any relationship with the AE (causality). Participants will have renal and liver function checked at screening and weeks 4, 12 and 26. They will also have 12-lead electrocardiograms (ECGs) performed at the same intervals.

Sample size

50 patients will be randomised equally (1:1) between the active arm (the combination of metformin and clemastine) and the placebo arm. The primary analysis will be on an intention-to-treat on the whole study cohort.

In developing this trial, I worked with Dr Wendi Qian, now the trial statistician, to perform the sample size calculations. We powered CCMR-Two to detect an average reduction of 3.0 ms/eye in full-field VEP latency at week 26 between metformin + clemastine (research arm) and placebo (control arm). The common standard deviation in our power calculation was 5, based on the ReBUILD study,[277] which was approximately 4.1; we have added 20% (4.1x1.20 = 4.92) more variation. With a 5% significance level (two-sided), 80% power, and a common standard deviation of 5, 45 eyes in each arm are required to detect a difference of 3.0 ms/eye in the mean change of VEP at week 26 from baseline between two treatment groups (nQuery). Allowing for 5-10% of non-compliance,[442,443] that is 50 eyes per arm, it is therefore planned to recruit 50 patients.

These estimates are limited by their using a common standard deviation derived from another group's dataset. With a more heterogenous group of patients in CCMR One, the common standard deviation was 5.75. This trial, however, returned a statistically significant result as the effect size was an improvement of 4.06 ms. We have therefore decided to test the aforementioned assumptions in this sample size estimation: when 20 patients have had their primary endpoint data assessed, the

trial's Independent Data Monitoring Committee (IDMC) will review the data and advise whether a sample size re-estimation should be considered.

Assignment of interventions

Eligible participants will be randomly assigned to metformin + clemastine (active arm) or placebo (control arm) in a 1:1 ratio using a minimisation with random element method. The stratification factors used in the randomisation will be: age (≤40 vs >40 years), baseline MS DMT treatment category (1 vs 2), gender (male vs female) and disability (an EDSS step ≤4.0 vs >4.0). We have chosen these criteria to promote matching of baseline characteristics between the active and placebo arms. In contrast to CCMR One – in which all participants were on dimethyl fumarate – we have instead introduced DMT category as a stratification factor. This decision reflects the realisation of the recruitment challenge afforded by only including those on a single DMT, but a corresponding desire to ensure comparable proportions of participants on high-efficacy DMTs between the two groups; such a mismatch might affect the interpretation of the trial result. Additionally, in the knowledge that disability correlates with axonal loss,[449] while intact axons are the substrate of remyelination,[28] we have similarly introduced EDSS into our stratification.

A web-based central randomisation system will allocate the participant a trial ID and treatment code which will relate uniquely to a supply of IMP. Access to the web-based randomisation system will be via individual user accounts provided to the Principal Investigator (PI) and suitably trained and delegated members of the research team. Immediate allocation of treatment will be performed, with documentation of the decision sent in a confirmatory email.

Throughout the trial investigators and participants will be blinded to the treatment allocation. The blind will be protected by means of: identical appearance of tablets for active and placebo drugs, equal numbers of tablets prescribed to participants in each arm, coded drug supplies being provided to the clinical trials pharmacy, and the randomisation list being held securely from the investigator's team (with provision for

unblinding in emergencies if required). An additional layer of blinding is added by the anonymisation of VEPs and MRIs, such that they are analysed without reference to any participant information.

Statistical analyses

Descriptive statistics

The pathway of participants through the trial will be summarised in a CONSORT flow diagram. Baseline characteristics will be summarised by treatment group, using summary statistics (mean, SD, median, IQR, maximum and minimum) for continuous variables and frequencies and percentages for categorical variables. Proportions of patients with missing and non-missing follow up data will also be reported in each treatment group.

Primary VEP outcome measure

The primary outcome, the mean change in the P100 latency of the full-field VEP between baseline and week 26 for eyes with a baseline latency of ≥118 ms, will be compared between the active and placebo arms using a mixed effects model applied with random effects for participant and eyes within participant, adjusting for fixed effects for baseline VEP latency and minimisation variables: age, gender, DMT category and baseline EDSS. The primary analysis will be on complete cases of patients with baseline and week 26 data.

Secondary outcome measures

Using the same method as for the primary VEP analysis, the mean difference in multifocal VEP latency from baseline to 26 weeks will be compared between the active and placebo groups. The lesion-level MTR analyses will use linear mixed models for lesions nested within patients, with patient random intercepts; these models regress lesion MTR on the same prespecified covariates but with lesion-subgroup interaction terms to estimate lesion-subgroup specific treatment differences and test for variation between these differences.

Exploratory outcome measures

If the changes in EDSS, colour vision and Sloan LCLA are found to be normally distributed, comparisons will be made between active and placebo arms using linear models, adjusting for baseline values and minimisation variables. The change in EDSS, colour vision or LCLA will be entered into the model as a continuous variable, as will age and baseline value; gender and DMT category will be included as a categorical variable. If normality cannot be assumed, unadjusted non-parametric Mann-Whitney U tests can be used to compare active treatment to placebo.

Safety analyses

The safety analyses will be based on all participants who received at least one IMP dose. Summary tables will be presented for incidence rates (number of patients with at least one event) of AEs and serious adverse events (SAEs), categorised according to the Medical Dictionary for Regulatory Activities (MedDRA) classification. The AEs that caused treatment modifications will be listed.

Ethics and dissemination

Before the start of the trial we obtained ethical approval from the Nottingham NRES Committee (21/EM/0120), which included endorsement of the trial protocol, participant information sheet (PIS), informed consent form (ICF), advertisements, and GP information letter. We will additionally seek approval before implementation of any substantial amendment. The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of good clinical practice, the protocol and applicable local regulatory requirements and laws.

Informed consent

All participants of CCMR Two will be required to provide written informed consent prior to any trial-specific procedures are carried out. Participants will agree to their records being inspected by the trial team, regulatory authorities and representatives

of the sponsors. They will also agree that their GP be informed of their participation in the trial and be informed of any abnormal investigations during the trial.

Confidentiality

All investigators and trial site staff involved in CCMR Two will comply with the requirements of the General Data Protection Regulation (GDPR) 2018, Data Protection Act 2018 and trust policy with regards to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles. The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant ID number on the case report form and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel.

Copies of the raw data from the full-field VEP, MF-VEP and saccadometry testing will be stored on internal University of Cambridge Secure Data Hosting Service (SDHS). The SDHS provides a dedicated network provides a dedicated network for storing sensitive personal data. Access to this data will be restricted to authorised members of the trial team via a two-factor authentication system.

Data access

The chief investigator will have full access to the final data set following completion of the analysis by the trial statistician. The datasets generated during the study will be made available with publication from our website: https://www-neurosciences.medschl.cam.ac.uk/jones-coles-group/ccmr-one-bexarotene-trial-datasets/.

Dissemination policy

The results of the trial will be released either at oral presentation or study publication, after a dissemination plan is agreed with the trial steering committee. Summaries of results will also be made available to participants by means of a newsletter (communicated by email) and by means of an informal meeting with the trial team

(virtual or in person). Authorship decisions will be guided by uniform requirements for manuscripts submitted to medical journals (www.icmje.org).

Patient and public involvement

Patient and public involvement (PPI) has been valuable the development of this research and will continue to be involved in the management of the trial. As part of the design and preparation for the study we have shared our protocol and participant information sheet with ten people with MS who have provided constructive feedback. People living with MS from the MS Society's Research Network were also involved as part of the peer review process undertaken during the funding application. All have been in agreement with the trial design and thought that both documents outlined what would be expected of its participants and would be acceptable to people living with MS. Contributions from our PPI group have specifically led to: (i) a change to the visit schedule so that not more than one electrophysiological investigation (VEP or saccadometry) occurred on the same date; (ii) changes to the phraseology of the PIS to more clearly explain the overall aim of the trial, and the rationale for its selection criteria; and (iii) the consideration that the burden of the trial assessments were acceptable in view of the information they would provide both for this trial and those that might follow it. In the management of the trial, two people with MS from the MS Society's research network will serve as members on the trial steering committee, ensuring the views of people living with MS remain linked to our research throughout the study.

Discussion

While several clinical trials have deployed drugs capable of enhancing differentiation of the OPC,[277,285,290] the demonstration that metformin can reverse age-related changes in OPCs that prevent them from responding to such factors is an important development.[100] Consequently, there is a compelling rationale to test metformin, alongside pro-differentiation agents such as clemastine, in clinical trials of people with MS.

CCMR Two will test the hypothesis that metformin and clemastine can enhance remyelination in people with MS using both visual evoked potentials and magnetization transfer ratio imaging; it is anticipated that in this way we will be able to detect the functional and structural consequences of remyelination with a sample size feasible for a single centre study. We have applied our experience from the CCMR One trial – which showed that lesion remyelination in response to RXR agonism was dependent on its location and baseline tissue integrity – and optimised our analyses to be sensitive to remyelination in those lesions that are more demyelinated at baseline and those that are located in the grey matter. We have additionally introduced selection criteria to yield a cohort that we expect is most likely to have a measurable between-group response in this trial setting.

There are, however, limitations to our chosen trial design. A particular challenge to translating promising preclinical research into remyelination trials is uncertainty about the optimum outcome measures to employ.[450] Our approach for CCMR Two has been to prioritise the visual evoked potential, given it has been directly confirmed to reflect myelin status in chronically demyelinated optic nerves[418] and has shown significant effects in three previous clinical trials,[277,285] but to also include the MTR analyses that we think will be most sensitive to remyelination; in CCMR One, perhaps the most compelling evidence of a biological effect of bexarotene was the alignment between the imaging and electrophysiological results. We have also included several techniques that might be more sensitive to, or represent the downstream consequences of, remyelination: multifocal VEP,[360] OCT,[372]

saccadometry,[391] colour vision,[357] and LCLA.[384] However, all outcome measures in this field are essentially exploratory and several of this trial's limitations stem from this point. First, in selecting only relapsing remitting patients with visual pathway demyelination and focussing on remyelination of this particular tract, there follows uncertainties about the generalisability of results to the wider MS population. Therefore, if CCMR Two did return a positive result, a further trial with a definitive disability outcome would be needed. Second, the sample size calculation is confounded by doubts in what constitutes a clinically important effect size and uncertainty in the variation in effects we might observe; we hope to mitigate against the latter point through the IDMC mid-point review of the sample size calculation.

An additional limitation is the interpretation of a positive result. Would the primary effect be primarily attributable to the action of metformin, clemastine, or is there evidence of synergy between the two? There are pro-differentiation factors already present in demyelinated lesions[210] and metformin alone might be sufficient to achieve remyelination. Meanwhile, the modest effect of clemastine on the VEP in ReBUILD, yet no MRI improvement,[277] might indicate a mild pro-differentiation effect that can be better 'unlocked' by co-administration of metformin. CCMR Two is not designed to test these questions; the objective is to see if the remyelinating effect seen in animals translates into humans. Dissecting the different contributions of each drug and interrogating the possibility of synergy would require a much larger study, which would then demand a multi-centre design, which then poses a particular challenge to using electrophysiological outcome measures.[358,450] Questions would also remain as to how to deploy a remyelination treatment, such as whether it should be given continuously or in short pulses.

Finally, it should also be acknowledged that remyelination failure may not be entirely due to a block to OPC differentiation; recent evidence has suggested that pre-existing oligodendrocytes can contribute to remyelination in humans[237,238]. Therefore, the effect of metformin and clemastine, which would only target the abovementioned endogenous OPC differentiation step, may not address

remyelination failure across a population of lesions, which may have heterogenous reasons for failing to repair.

In spite of this, CCMR Two represents an important step towards identifying a treatment capable of directly protecting neurons from degeneration, outside of the indirect effects of immunomodulatory disease modifying treatments. Further, as these drugs are widely used and known to be safe, this approach could then be readily deployed in confirmatory and phase III trials. And, if no treatment effects are shown, we are confident that the trial will have legacies that will lead to improvements in trial design and outcome measures.

Chapter 6: Neuroprotective treatments for progressive MS

A systematic approach to selecting licensed drugs for repurposing in the treatment of progressive multiple sclerosis

Abstract

Although substantial progress has been made in the development of effective treatments for relapsing-remitting MS, these disease modifying treatments do not address the multifaceted pathophysiological mechanisms of progression. The lack of success in therapeutic development for progressive MS has led to interest in novel approaches such as drug rescue and repurposing. Techniques are therefore required to enable rational selection from a wide range of candidates, in order to maximise the chance of successful clinical development.

In this body of work, we sought to establish a rigorous, expert-led, evidence-based approach to the evaluation of licensed drugs for repurposing and testing in clinical trials of people with progressive multiple sclerosis. We long-listed licensed drugs with evidence of human safety, blood-brain barrier penetrance, and demonstrable efficacy in at least one animal model, or mechanistic target, agreed by a panel of experts and people with MS to be relevant to the pathogenesis of progression. I systematically reviewed the preclinical and clinical literature for each compound, condensed this into a database of summary documents, which were presented to the rest of the treatment selection panel for short-listing by scoring each one of them. Drugs were then evaluated for immediate use in a clinical trial, following which our selection was sent to be scrutinised by a final independent expert review.

From a short list of 55 treatments, we recommended four treatments for immediate testing in progressive MS: R-α-lipoic acid, metformin, the combination treatment of

R-α-lipoic acid and metformin, and niacin. We also prioritised clemastine, lamotrigine, oxcarbazepine, nimodipine and flunarizine.

Contribution statement

I was a member of the treatment selection group and worked alongside the rest of the panel in designing a process for shortlisting treatments. After the pilot stage of this process, I volunteered to take on a more active role, in particular addressing the heterogeneity in the levels of detail in the drug CVs, alongside the MS Society's Kayla Vuong. I worked to create our prioritised list with the rest of the group and attended the meetings to ultimately formulate our final list. I was asked by the group to document our methodology: I wrote the first draft of the manuscript, compiled all of the edits, submitted this for publication, and made the needful revisions.

Background

The expanding repertoire of anti-inflammatory disease modifying treatments (DMTs) contrasts with a paucity of effective therapies for the 15% of people that present with progressive disability (primary progressive MS; PPMS), and indeed the 80% of RRMS patients who subsequently develop progression (secondary progressive MS; SPMS).[191] While ocrelizumab and siponimod have shown modest benefits in phase III trials,[24,25] most immunotherapies have failed in non-active progressive disease. Finding drugs to treat progression remains the greatest unmet need for people with MS.

The reasons for the lack of an effective therapy for progressive MS are multifaceted. The pathophysiology of progressive MS is poorly understood (reviewed in [26]), and there is no animal model that accurately mimics the entirety of the disease. So, new target and drug discovery are challenging. Drug repurposing is attractive, with fewer hurdles before reaching clinical trials, but the rationale behind drug selection needs to be carefully considered.[451,452]

In 2011 the MS Society sponsored an initiative to choose licensed drugs to be trialled in secondary progressive MS.[453] Only oral treatments with a putative action against neurodegeneration were considered. Highest priority was given to drugs that had been tested in MS, Alzheimer's disease, motor neuron disease / amyotrophic-lateral sclerosis, Parkinson's disease and/or Huntington's disease. Clinical and laboratory data from each drug were brought, in a standard template, to a panel composed of people with MS, and experts in animal models, disease biology, clinical trial design and systematic review. The final panel treatment selection was: riluzole, amiloride, fluoxetine, ibudilast, oxcarbazepine, pirfenidone and agents of the polyunsaturated fatty acid (PUFA) class (including lipoic acid). Of these, both ibudilast and lipoic acid have since shown efficacy in progressive MS in phase II trials.[201,454] Meanwhile, the MS-secondary progressive multi-arm randomisation trial (MS SMART) tested riluzole, amiloride and fluoxetine versus placebo in 445 people

with SPMS.[346] Unfortunately, no treatment effect on brain atrophy (percentage brain volume change) was seen over 2 years.[119]

In 2018, the MS Society set up an expert consortium (Figure 6.1) to select treatments and design a new phase of drug trials in progressive MS utilising a novel adaptive methodology. I joined the treatment selection component of this consortium, with the objective of augmenting the previous strategy with an expert and mechanism-led approach. I worked with the rest of the panel to design a process for shortlisting treatments, which required our compiling a list of drugs of seminal interest, my preparing a database of summary documents for each, before we created our prioritised list by consensus agreement as a group.

Methods

Pilot stage of treatment selection

The original treatment selection group included 10 scientific members (specialist multiple sclerosis clinicians, laboratory scientists, people with experience of the pharmaceutical industry) and two people with MS. The latter were selected from the MS Society's Research Network (RN): a group of people living with, or caring for, someone with MS.

At the first meeting of the treatment selection group in January 2018 we agreed the following principles of treatment selection: the highest priority would be given to safe licensed drugs acting on pathological mechanisms thought to be relevant to progression in multiple sclerosis, including remyelination; to drugs which cross the blood-brain barrier; and those that had demonstrable efficacy in at least one relevant animal model. Experience of the drug's use in MS or any other neurological illnesses was considered but did not weight treatment choice. Immunotherapies, such as B-cell depleting drugs, were excluded, given the considerable industrial investment in this area. The agreed mechanistic areas were: (i) energy, blood flow and mitochondria; (ii) the neuron and axon; (iii) sodium channels; (iv) microglia and astroglia; (v) intrathecal B cells and plasma cells; (vi) demyelination and myelin

repair; and (vii) antioxidants. It was also agreed that the process of drug selection should be iterative, using a modified Delphi method, led by expert opinion within treatment selection group, while at each stage independent expert input would be sought.

We then convened an international treatment selection workshop, held in London in April 2018. Leading experts from the research community gave a series of talks in each mechanistic area and were asked to suggest drugs for consideration. We also invited representatives of the Cure Parkinson's Trust, the Alzheimer's Society, Motor Neuron Disease Association, Parkinson's UK, and Medicine Discovery Catapult (MDC), who had undertaken drug repurposing programmes within their own disease area.[455,456] We resolved to draw up a template (a "drug CV") for each compound based on the Cure Parkinson's Trust linked clinical trials initiative dossier model. These documents included information on pharmacodynamics, pharmacokinetics, mechanism of action, and evidence-base in vitro, in vivo, and in clinical trials (Table 6.1). This CV condensed and systematised the literature on each drug into an accessible summary manuscript; a drug CV for each potential treatment would be completed by at least 2 members of the treatment selection group.

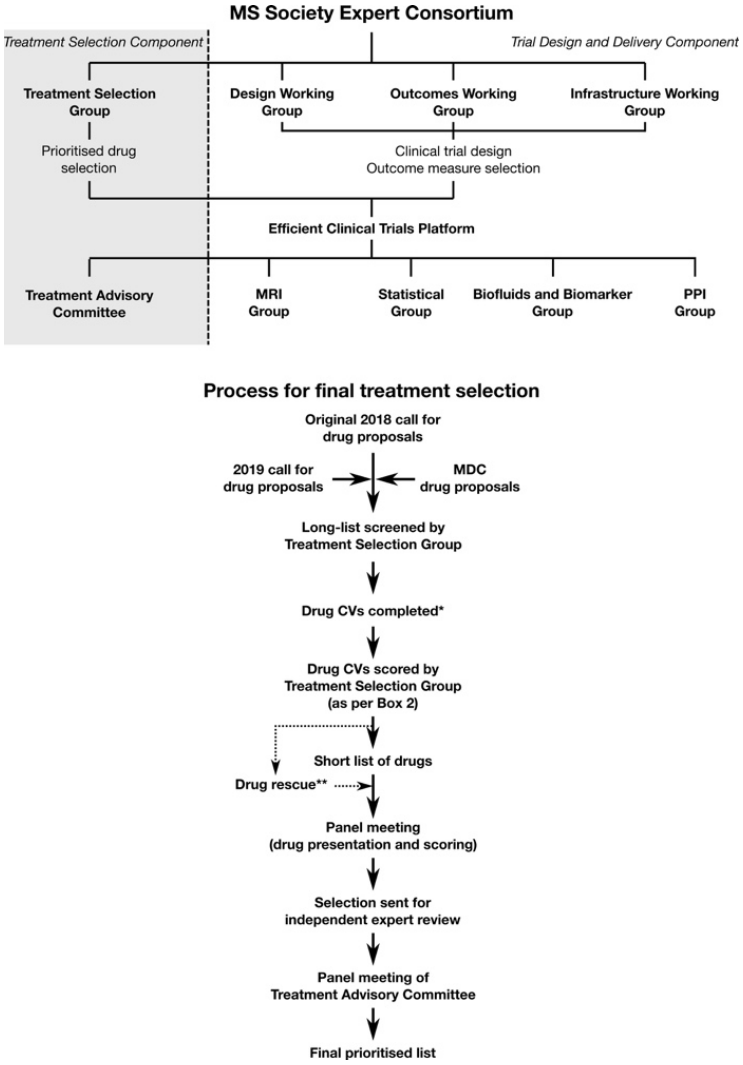

Figure 6.1. *Above* Summary of the UK MS Society's expert consortium for progressive MS clinical trials, which has been set up to test treatments in an adaptive platform trial, termed the efficient clinical trials platform. The treatment selection group and treatment advisory committee were responsible for formulating the prioritised list of drugs to enter the clinical trial. *Below* Flow chart of the procedures undertaken during the final round of treatment selection by the treatment selection element. *Drug CVs were completed by 4 members of the treatment selection group – 2 with a scientific background and 2 MS specialist clinicians. **Drugs failing to reach the short list of drugs on account of a low score, could be added back for consideration at the panel meeting if reasons were proposed by a member of the treatment selection group and its rescue approved by majority vote. MDC: medicines discovery catapult; PPI: patient and public involvement.

Table 6.1: Information recorded in the drug CV

Summary information

- Drug name
- Regulatory status
- Mechanistic target
- Dose for human use (and appropriateness for MS)
- Key safety concerns
- Intellectual property
- Outstanding critical issues
- Overall evaluation

Absorption, distribution, metabolism, excretion, pharmacokinetics and pharmacodynamics

- Chemical structure
- Molecular target
- Pathway affected
- Human pharmacodynamics
- Human pharmacokinetics
- Blood-brain barrier penetrance
- Route of administration
- Licenced indication
- Dose for licenced indication
- Dose suitability for MS
- Known, or anticipated, drug-drug interactions

Scientific rationale

- Efficacy in *in vitro* models
- Efficacy in *in vivo* models
- Efficacy for primary indication
- Efficacy in people with MS (if applicable)
- Particular subgroups of people with MS likely to benefit (if applicable)

Safety

- Animal safety issues
- Therapeutic ratio (if known)
- Safety record in humans
- Safety record in people with MS
- Monitoring requirements
- Any particular drug-drug interaction that would limit use in MS

Landscape review

- Is there active pre-clinical research on the use of this drug in MS? Where?
- Has the Progressive MS Alliance prioritized this drug?
- Are there any relevant trials listed on clinical trials databases?

There was then a call for suggestions for repurposed drugs to members of the committee, clinicians, experts from the wider MS research community, people with MS, and the public, via a web-based system that was advertised to the MS Society's mailing lists. Contributors were prompted to describe the scientific rationale for their proposed intervention. After four months, the call was closed, a long list of drugs was compiled, and drug CVs completed for each.

The scientific members of the committee scored each drug CV according to an agreed system prioritising safety and efficacy (Table 6.2A). Members of the MS Society's research network also scored each drug, for ease of administration, tolerability, safety and monitoring requirements based on the drug CV and the European Medicines Agency's (EMA) patient information leaflets (Table 6.2B). The scores were collated before a second face-to-face meeting.

At this meeting, in September 2018, the treatment selection group (voting) members were joined by new members of the research community and research network (invited attendees), to provide a fresh perspective on the drug list. Each drug was presented, discussed, and given an overall score (between 0 lowest and 5 highest). The results were further reviewed and discussed, before all attendees ranked their top 5 drugs, which resulted in a list of seven prioritised drugs.

In parallel to the pilot stage of treatment selection, the MS Society commissioned Medicines Discovery Catapult (MDC) to independently identify licensed drugs that might impact progressive MS. This was undertaken to scrutinise our long list of drugs which had been compiled through the aforementioned mechanism of drug proposals. MDC searched for all ongoing, or completed, trials in people with MS to identify drugs being tested for any type of MS. They then characterised their molecular targets and sought other compounds that were predicted to impact these targets. We pruned the list of immunotherapies and symptomatic drugs, as well as those that did not cross the blood-brain barrier, and any not on the original long list were added for consideration during the final stage of treatment selection.

Table 6.2: scoring system for shortlisting drug CVs
A) For scientific members of the panel **Safety** – Are the safety data for the treatment satisfactory? To include any regulatory warnings, adverse events, drug-drug interactions, therapeutic index, and safety profile. (Score 0-2) **Efficacy** – Do we have sufficient evidence that the treatment is likely to be effective in slowing progression? To include *in vitro* and *in vivo* experimental models, blood-brain barrier penetration, along with human data where available. (Score 0-2) **Overall evaluation - Priority level for the treatment** (select one) a. Licensed drug, ready for a phase 2 trial in MS, high priority b. Licensed drug, ready for a phase 2 trial in MS, low priority c. Licensed drug, with critical issues to be resolved before a phase 2 trial in MS d. Interesting drug, with considerable pre-clinical work to be done e. Poor scientific rationale: not to be prioritised
B) For research network members (people with MS) **Administration** – is the method of taking the drug acceptable? To consider whether it is a tablet, injection or infusion as well as how often it needs to be taken. (Score 0-2) **Side effects and risks** – is the safety of the drug acceptable? To consider both the immediate side effects and risks as well as the long term. (Score 0-2) **Overall evaluation - Priority level** (select one) a. I would take this drug even if it only moderately slowed the progression of my MS. b. I would take this drug if it stopped the progression of my MS. c. I would not take this drug even if it stopped the progression of my MS.

Final stage of treatment selection

The treatment selection group appointed new members, and some original members left, leaving 13 scientific and 6 research network members. A renewed call for drug proposals was opened, and the newly formed group reviewed any new suggested compounds, the original long list of drugs considered during the pilot stage, and those generated by Medicines Discovery Catapult, resulting in a new long list of 29 drugs. Each of these had a drug CV compiled or updated by myself and another with a scientific or clinical background. Myself and the other 12 scientific members of the treatment selection group then scored each drug CV according to our simplified scoring system based on safety, efficacy and an overall assessment of priority (Table 6.2). Similarly, 6 research network (RN) members of the treatment selection

group and an additional 10 invited RN members scored between 5 and 10 of the drug CVs, with additional access to the EMA-approved patient information leaflet, such that at least 5 scores were recorded for each drug. The highest ranked 12 drugs from the collated scores formed the shortlist for a third face-to-face meeting in September 2019 of the treatment selection group, alongside a new group of invited experts and people with MS.

Members of the treatment selection group had the option to rescue a low scoring drug in advance of the meeting by presenting a case for its inclusion and it being accepted by majority vote. For the meeting, each drug was presented by one scientific and one research network member, who focussed on the scientific case and attractiveness to people with MS, respectively. Drugs were then scored out of 5 and the resulting ranking discussed before each attendee individually ranked up to 5 drugs that they felt ready for use in a clinical trial.

External scrutiny of treatment selection

The drug CVs of the treatments recommended by this meeting, and the two highest scoring drugs in the sodium channel antagonist class, were sent to 4 independent international MS experts outside the UK to achieve a further layer of scrutiny of the decisions and to elicit any information on the drugs that was not publicly available. Their comments were collated and considered alongside the outcome of the final treatment selection meeting by the Treatment Advisory Committee (Figure 6.1). This committee advised on the final drug selection for the MS Society's Efficient Clinical Trials Platform, which is intended to evaluate repurposed treatments quickly and affordably. This committee comprised 6 scientific members and 3 people affected by MS. They assessed the prioritised list on the basis of scientific evidence, but also in the context of other trials known to be going ahead elsewhere. They also scrutinised drug mechanisms and whether the chosen trial design and outcome measures would allow detection of treatment effects. This facilitated a final decision to be made for the drugs to be tested in a platform trial (Figure 6.1).

Results

Pilot stage of treatment selection

44 treatments were proposed during the 2018 call for drug suggestions, with at least one believed to act on each of the target mechanisms. 35 were deemed by the treatment selection group to have sufficient scientific rationale for consideration, and drug CVs were completed by myself and other scientific members. Each was then scored, prioritising considerations of efficacy and safety as detailed in Table 6.2, leading to a shortlist of 19 compounds to be discussed face-to-face in September 2018. At that meeting, each drug was presented and discussed before being scored again, collated separately for the members of the treatment selection group (voting members) and the invited attendees (experts and people affected by MS) (Figure 6.2). After open discussion of these scores, the treatment selection group members ranked their preferred 7 drugs.

During this pilot stage we learned that the drug CVs were effective, but needed more consistency in authorship to promote comparable levels of detail in each CV, with multiple contributors from different backgrounds to each to encourage impartiality in the presentation of the literature for each compound. We also reflected on the valuable contributions from people affected by MS, who were in a unique position to weigh the safety and tolerability of each drug and consider the level of benefit they would require to take the proposed treatment for their MS. The group resolved that more research network members should be invited onto the treatment selection group to maximise representation of different viewpoints from within the MS community and to share the burden of scoring CVs and presenting drugs at meetings beyond the 2 original members.

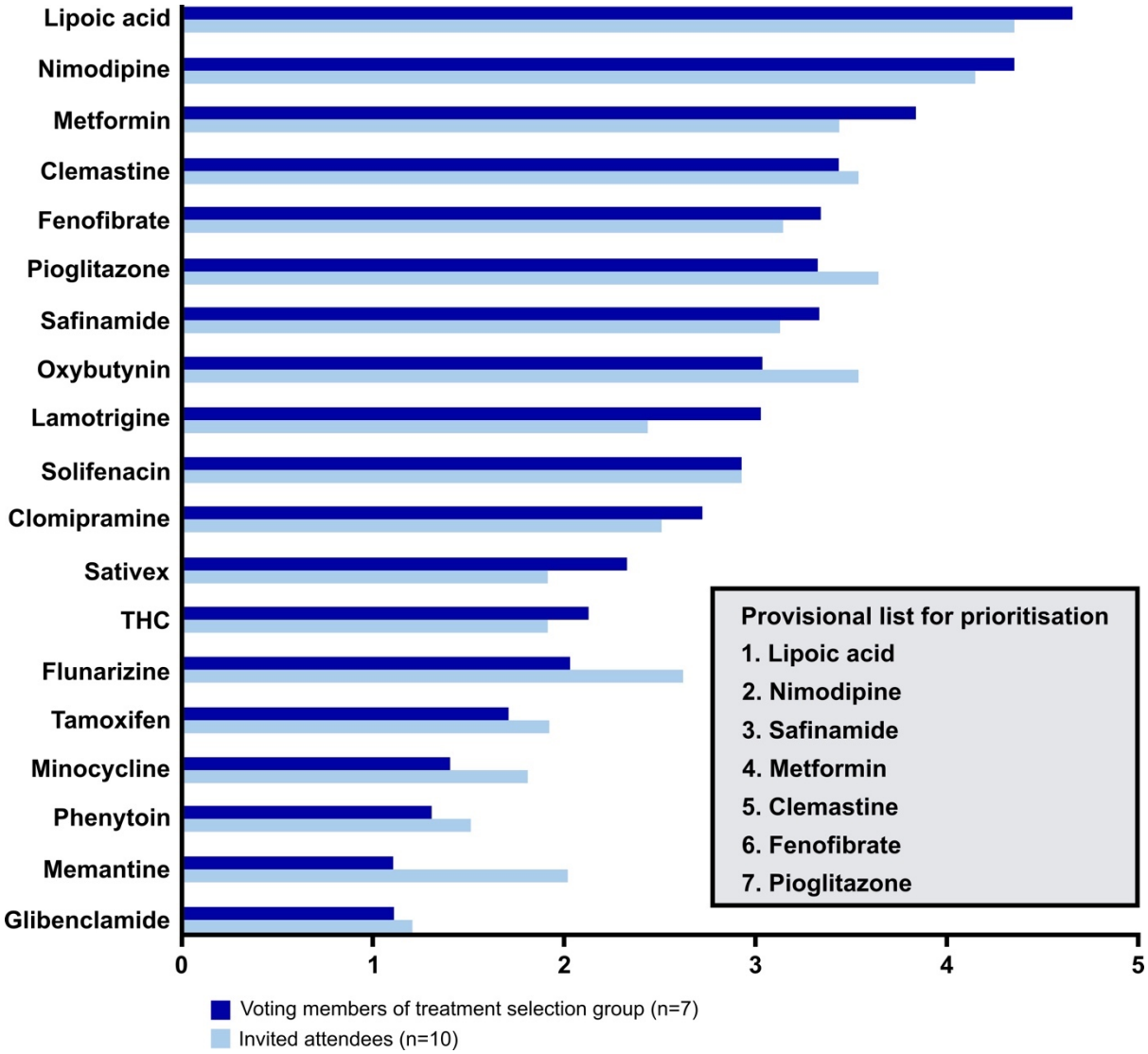

Figure 6.2. Outcome of the pilot screen of candidate interventions. Mean scores (out of 5) of each drug by voting members of the treatment selection group and invited attendees are displayed in descending order. Inset: the provisional list for prioritisation agreed by the voting members of the committee.

Final stage of treatment selection

MDC identified 320 licensed drugs which had a mechanism similar to a drug that had been tried in multiple sclerosis.[457] Once immunotherapies, drugs which did not cross the blood brain barrier and duplicates were removed, guanabenz and trazodone remained from this list. These were added to the 44 treatments that had emerged from the pilot phase. During the renewed call for proposals in 2019, new members of the treatment selection committee and outside experts contributed these new suggestions: domperidone, benztropine, prednisolone, ibudilast, spironolactone, oxcarbazepine, hydroxychloroquine, niacin and the combination of metformin and R-α-lipoic acid. This long list of 55 treatments was screened by the new treatment selection group, and 28 drugs and one combination therapy were chosen to have comprehensive drug CVs completed by myself and at least one other.

12 scientific members of the group scored all 29 drug CVs and 16 research network (RN) members (6 of which were members of the treatment selection group) scored up to 10 of the drug CVs, with additional access to the EMA-approved patient information leaflet, such that 5 research network scores were recorded for each drug. The scientific scores were ranked and 13 drugs and 1 combination treatment (metformin and R-α-lipoic acid) were short-listed. If a scientific member disagreed with a drug excluded at this stage, they were able to make a case for its inclusion to the group and add to the shortlist by majority vote. Flunarizine and lamotrigine, which had initially been excluded from the list of 12 at the CV scoring stage, were re-added to the list in this way.

The 14 shortlisted treatments were discussed and scored, one by one, at a face-to-face meeting of the treatment selection group and invited attendees. The collated scores (Figure 6.3) were then discussed and debated before the treatment selection group ranked up to 5 drugs, which were ready for immediate use in a phase II clinical trial. The final shortlist list of drugs were, in order of preference: R-α-lipoic acid, metformin, the combination treatment of R-α-lipoic acid and metformin, and clemastine. We considered that niacin, flunarizine, and nimodipine were particularly promising, but the treatment selection group felt they needed more pre-clinical work.

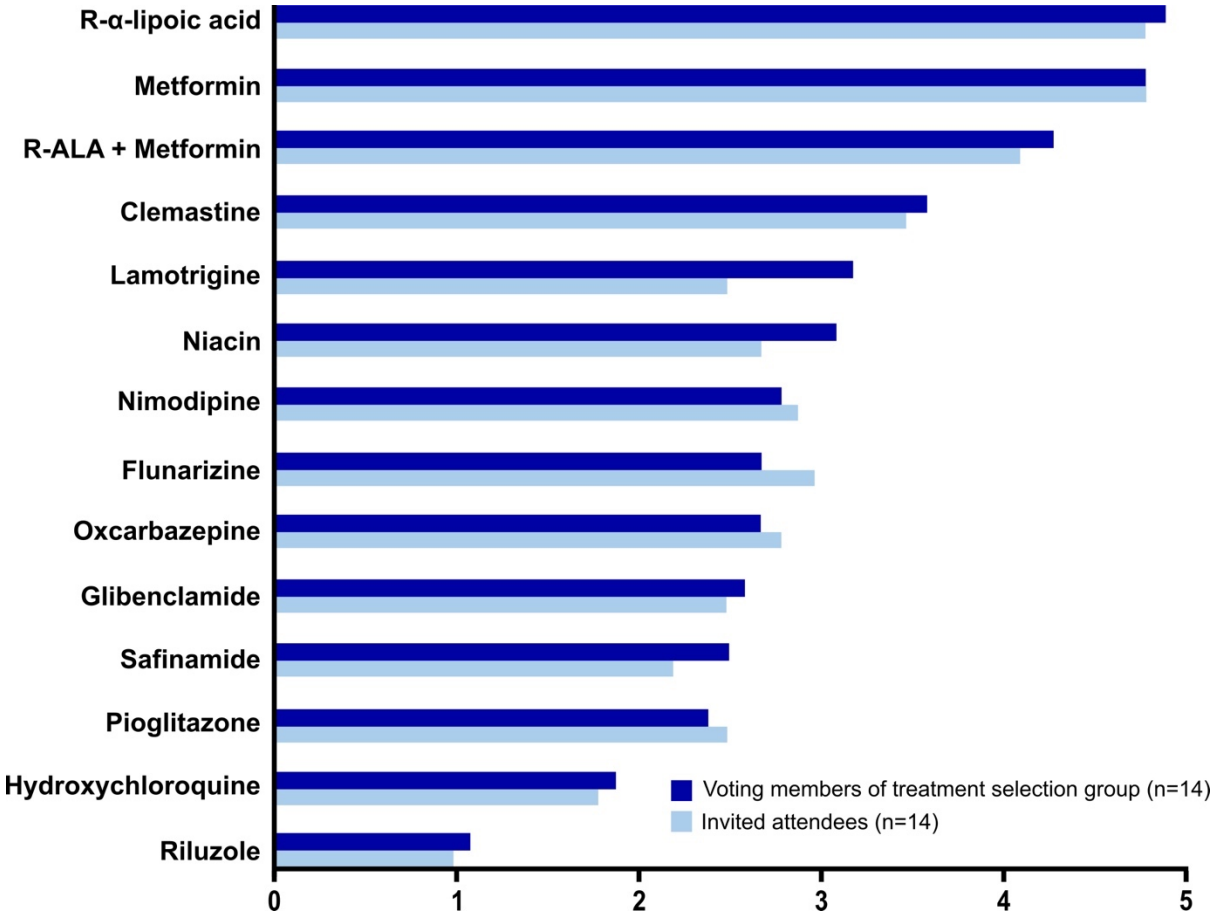

Figure 6.3. Outcome of the final meeting of the treatment selection group during the final stage of candidate screening. The mean scores (out of 5) for the 14 shortlisted compounds presented at the meeting are divided into those awarded by voting members of the panel and invited attendees.

This selection, in addition to the 2 highest scoring sodium channel antagonist drugs (lamotrigine and oxcarbazepine), were sent to 4 independent expert reviewers. They scored each compound on safety and efficacy and ranked the drugs by priority level. They were also asked to provide information on any of these drugs that was not publicly accessible. The results of this procedure were considered by the Treatment Advisory Committee of the MS Society's Efficient Clinical Trials Platform (Figure 6.1), and a final order of prioritisation was made (Table 6.3). The top 4 were recommended as the most promising for clinical evaluation. The pathway of each drug through these procedures is summarised in Figure 6.4.

Final list of drugs for prioritisation	Mechanism of action
1. **R-α-lipoic acid**[a]	**Dietary supplement, approved in Germany for diabetic neuropathy;** anti-oxidant, anti-inflammatory, and neuroprotective[454,458,459]
2. **Metformin**[b]	**Anti-hyperglycaemic agent used for type 2 diabetes mellitus;** anti-inflammatory[440] and promotes remyelination[100] and neuroprotection[460]
3. **R-α-lipoic acid and metformin combination**	**Mechanisms as above;** complimentary mechanistic targets and neuroprotective in combination[461]
4. **Niacin**[c]	**Anti-hypercholesterolaemic drug;** promotes oligodendrocyte proliferation,[462] remyelination,[423] and neuroprotection[463]
5. **Clemastine**	**Antihistamine used for allergic rhinitis;** off-target anti-muscarinic (M1) action which promotes oligodendrocyte progenitor differentiation and remyelination[246,277]
6. **Lamotrigine**	**Sodium channel antagonist widely used as an anticonvulsant;** neuroprotective effects[464]
7. **Oxcarbazepine**	**Sodium channel antagonist widely used as an anticonvulsant;**; neuroprotective effects[465]
8. **Nimodipine**	**Calcium channel antagonist used to treat vasospasm in subarachnoid haemorrhage**; promotes remyelination, neuroprotection,[466] and restores CNS perfusion and oxygenation[467]
9. **Flunarizine**	**Migraine prophylactic;** neuroprotective effects[468]

Table 6.3. Final recommendations of repurposed interventions for clinical testing in progressive MS. The top 4 were determined to be the most promising for clinical evaluation. [a]1200 mg/day, [b]1 gram twice daily, starting at 500mg twice daily, [c]750mg twice daily of slow release formulation of Niaspan.

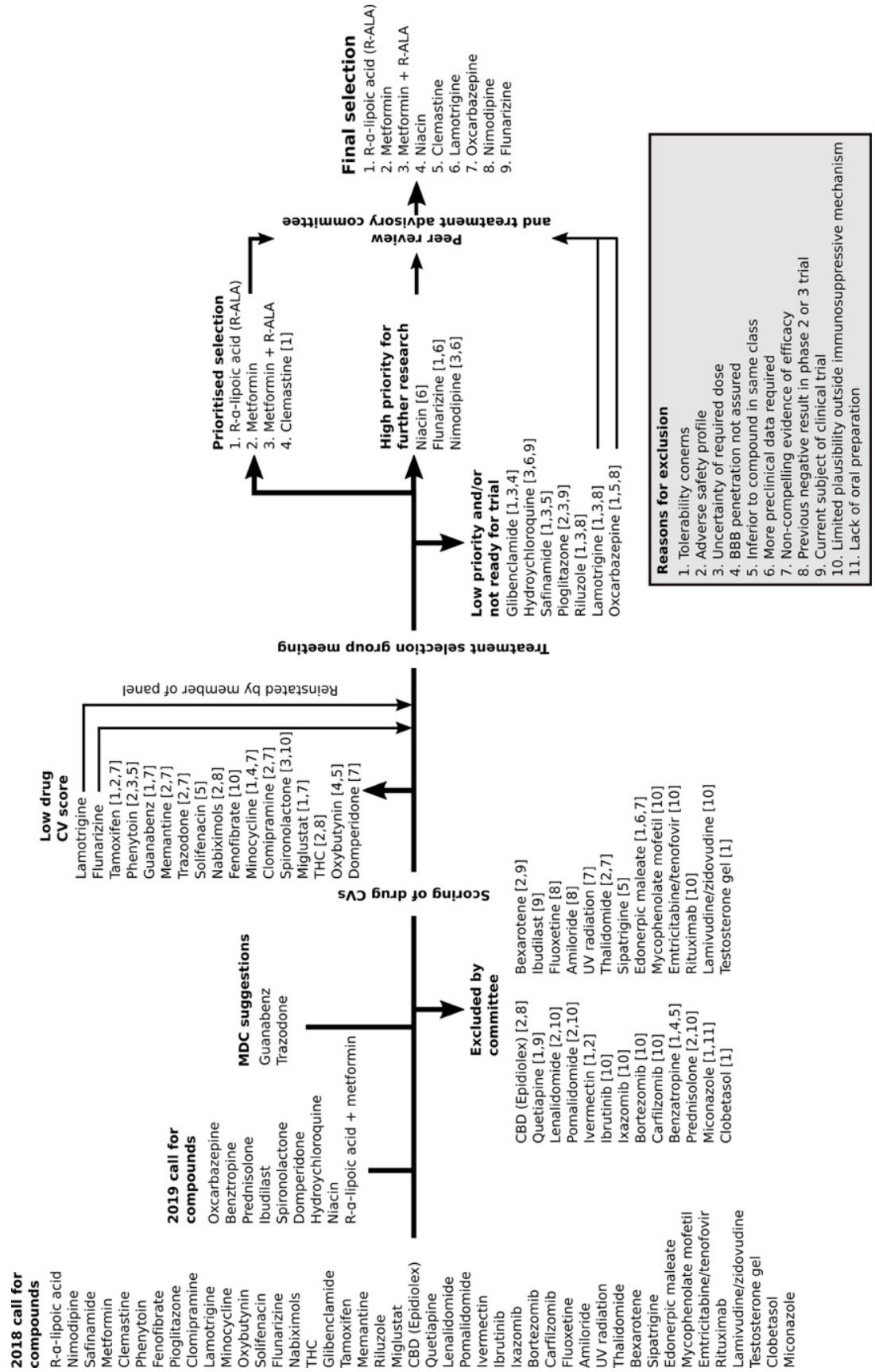

Figure 6.4. Summary of the pathway of each drug through the treatment selection process to yield a final prioritised list of drugs.

Discussion

The pathogenesis of progressive MS is complex, multifaceted and poorly understood. As with many other neurodegenerative diseases, there are no licensed treatments. This remains the greatest unmet need for the more than 2.3 million people affected by MS globally.[469] Placed in context of the high cost, long time, and high attrition rate from target selection to regulatory approval via conventional pathways, there are compelling reasons to explore opportunities provided by drug repurposing. This nevertheless presents a substantial challenge. The myriad reasons for the prior failure to find an effective treatment remain,[26] and the optimum process for selection of drugs to progress to repurposing clinical trials are not standardised. Procedures for synthesising experimental and clinical trial data to enable rational drug selection are required to maximise the chance of successful clinical development.

The UK MS Society Clinical Trials Network was initiated in 2007 and commissioned key underpinning work including a review of animal and human data on promising drugs. Given the mechanistic overlap between SPMS and other neurodegenerative disorders (namely Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis), their strategy centred on a systematic review and meta-analysis of clinical and preclinical data for agents previously tested in these illnesses.[453] The ensuing list prioritised ibudilast, riluzole, amiloride, fluoxetine, pirfenidone, oxcarbazepine and agents of the polyunsaturated fatty-acid (PUFA) class. Ibudilast and lipoic acid proved successful at phase II,[201,454] but unfortunately riluzole, amiloride and fluoxetine did not reduce brain atrophy in the MS-SMART study compared to placebo.[119]

The MS Society's ambition in setting up its expert consortium (Figure 6.1) is ultimately to deliver a platform trial capable of testing multiple drugs at the same time (as with MS-SMART), across thousands of people with MS (as with MS-STAT2), with an adaptive method allowing switching participants between drugs if efficacy is not demonstrated. The treatment selection group, to which I contributed as outlined

here, was tasked with identifying the most promising treatments to go into the trial first. In parallel the trial design and delivery component have been independently designing the trial and setting up its infrastructure. All of these contributions will ultimately converge on the multi-arm adaptive trial, which will be led by Professor Jeremy Chataway; the MS Society recently announced the Optimal Clinical Trials Platform for Progressive Multiple Sclerosis (OCTOPUS).

Here I describe the rigorous, expert-led, evidence-based approach we took to the selection of licensed compounds for repurposing in clinical trials of people with progressive forms of MS, led by scientific and clinical experts as well as people with MS, involving repeated rounds of assessment, scoring, and independent peer review. We identified key biological mechanisms, performed an exhaustive literature search on identified drugs, and went through two cycles of shortlisting and prioritisation. We selected this strategy to retain the evidence-based approach of previous mechanisms of drug selection, but with added emphasis on expert opinion and independent expert review which, in our view, would enable our selection to be based on current scientific opinion and more readily identify barriers and knowledge gaps that might affect trials of the proposed compounds. A particular contrast between our strategy and that previously used was that we did not prioritise agents that had previously been subject of clinical trials of people with neurodegenerative illnesses and we required all candidates to have evidence of blood-brain barrier permeability. Other differences are summarised in Table 6.4.

It is noteworthy that our first ranked drug, lipoic acid, was also prioritised in the 2011 drug selection initiative, despite the contrasting methodologies. Three interventions – R-α-lipoic acid (R-ALA), metformin and niacin – and one combination preparation – of metformin and R-α-lipoic acid – were identified as being priorities for clinical evaluation in cohorts of people with progressive MS, and as having sufficient data to permit immediate entry into a phase II trial. Clemastine, lamotrigine, oxcarbazepine, nimodipine and flunarizine were also felt to be promising and ranked in order of priority.

R-α-lipoic acid is the R-enantiomer that makes up 50% of the racemic mixture (R and S) of lipoic acid, a dietary supplement approved in Germany for the treatment of diabetic neuropathies. It has previously been shown to be a potent antioxidant, have anti-inflammatory properties,[470,471] and reduce excitotoxic damage;[472] while the R enantiomer has superior pharmacokinetic, antioxidant and neuroprotective properties than the S enantiomer.[473] When given to 51 people with SPMS, it was shown to have a small benefit to brain atrophy.[454,474]

Metformin, a biguanide licensed for human use in type 2 diabetes, has previously been demonstrated to reduce inflammation in progressive and relapsing experimental autoimmune encephalomyelitis models,[440] is neuroprotective in models of glucose deprivation/reoxygenation[460] and, more recently, has been shown to reverse an age-associated barrier to the ability of oligodendrocyte progenitor cells to respond to differentiation factors and facilitate subsequent remyelination.[100] Additionally, it has previously been used in 20 people with MS, and demonstrated a reduction in the number of new or enlarging T2 lesions compared to placebo.[441] The complimentary mechanistic targets of metformin and R-ALA, as well as the potential for synergy,[461] led to the combination of the two featuring on our prioritised list.

Niacin, a nicotinamide adenine dinucleotide (NAD) precursor in use for the treatment of hypercholesterolaemia, has previously been shown to be protective against activated microglial-induced neurotoxicity[463] and to promote oligodendrocyte proliferation *in vitro.*[462] In line with these observations, it reduces axonal degeneration, delays progression, and increases oligodendrocyte proliferation in extrinsic allergic encephalomyelitis.[462,463] While ranked below clemastine by the treatment selection group, data that was unpublished at the time came to light during the treatment advisory committee review: niacin also enhances myelin phagocytosis by microglia, leading to increases in oligodendrocyte progenitor cell numbers and improved remyelination in mice.[423] Niacin has not yet been trialled in people with multiple sclerosis.

A particular strength of this methodology was the multiple layers of revision and review. By undertaking a pilot of treatment selection, we refined the procedures by which we evaluated the literature and assessed each compound to facilitate robust comparisons of agents with disparate mechanistic targets and safety profiles. We also ratified our procedures for drug identification by the work of Medicines Discovery Catapult, which generated a list of drugs of which only 2 had not previously been identified. Finally, by sending our list of prioritised treatments for external peer review we have better ensured scrutiny of both our methods and our selection.

	2011	2019
Method of drug identification	Thorough and systematic search of online databases (PubMed, ISI Web of Knowledge, Embase, Clinicaltrials.gov, Cochrane MS group)	Calls for recommendations from academics, clinicians, and people with MS. Systematic search of online databases by medicines discovery catapult
Previous clinical trial use	Previously used in a neurodegenerative disease including progressive MS, PD, HD, AD, and ALS.	Human safety data required only
Mechanistic targets	Excluded immunosuppressant mechanism of action. Combination treatments excluded.	Priority given to candidates targeting several mechanistic targets. Excluded those with *solely* immunosuppressant mechanism. Combination treatments accepted
Method of administration	Oral	Any method of administration
CNS penetration	Reviewed at selection meeting	Evidence of BBB permeability required at study entry
Safety	Excluded those with significant adverse effects associated with treatment.	Excluded those with significant adverse effects associated with treatment.
Method of selection	Systematic evaluation of publications pertaining to each candidate. Systematic review of experimental autoimmune encephalomyelitis (EAE) preclinical data for each candidate. Scrutiny of each drug by an international multi-disciplinary committee	Systematic evaluation of preclinical and clinical publications pertaining to each candidate. Formation of a database of drug CVs. Rating of these by scientific panel. Presentation and decision at international multi-disciplinary meeting
Input from people affected by MS	Patient representatives acting as external advisors	6 members of MS research network on voting panel. Scoring of drug CVs by at least 5 people with, or affected by, MS. Members of MS research network at treatment selection meeting.
Peer review	External advisors with a range of expertise including animal models, disease biology, clinical trial design, systematic review and patient representation.	Methodology and final treatment selection sent for external peer review

Table 6.4. Comparison between the current methodology and that previously used in 2011;[453] MS, multiple sclerosis; AD, Alzheimer's disease; ALS, amyotrophic-lateral sclerosis; PD, Parkinson's disease; HD, Huntington's disease; BBB, blood-brain barrier.

Chapter 7: The factors affecting interventional trial recruitment during the first wave of the SARS-CoV-2 pandemic in the UK

Abstract

During the first wave of the coronavirus 2019 (COVID-19) pandemic, I volunteered to join a multidisciplinary group from the University of Cambridge to rapidly set up, and subsequently recruit participants to, interventional trials (particularly the RECOVERY trial). During this time, I led a research project to analyse enrolment to treatment trials, to describe the barriers to and implications of, low recruitment rates ahead of further waves.

I led a prospective observational study of hospitalised patients with COVID-19 who were being assessed for eligibility to one of the RECOVERY, C19-ACS or SIMPLE trials across 5 centres in a respiratory NIHR network. In parallel, I analysed registered interventional COVID-19 trial data from the clinicaltrials.gov and ISRCTN databases on July 12, 2020 and combined these with population and modelling data taken from published reports from the UK government and MRC biostatistics unit.

2,082 consecutive admitted patients with laboratory-confirmed SARS-CoV-2 infection from March 27, 2020 were included in the cohort study. 430 (20.7%) proceeded to randomisation in one of the aforementioned trials. 82 (3.9%) declined participation, 699 (33.6%) were excluded on clinical grounds, 363 (17.4%) were medically fit for discharge, 153 (7.3%) were receiving palliative care and 153 (6.6%) lacked capacity. Analysis of trial registration data for COVID-19 treatment studies enrolling in England showed that by July 12, 2020, 29,142 participants were needed. With 111,037 people hospitalised with COVID-19 in England by that date, we determined that 22,985 people were potentially suitable for trial enrolment. We

estimated a UK hospitalisation rate of 2.38%, and that another 1.25 million infections would be required to meet recruitment targets of trials on going at that time.

We concluded that recruitment rates, study design, and proliferation of trials can limit the number, and size, that will successfully complete recruitment. We considered that fewer, more appropriately designed trials, prioritising cooperation between centres would maximise productivity in a further wave.

Contribution statement

While working for the Cambridge COVID-19 clinical research team, I led this study of trial recruitment across the five centres; I collected the data from Addenbrooke's and collated it with that from the others. I additionally analysed the trial registry data and built a collaboration with Dr Villar of Medical Research Council (MRC) Biostatistics Unit to strengthen our assessment of the gaps between the trial community's aspirations and delivery. I wrote the first draft of the trial manuscript, marshalled all of the comments/edits from the authorship, and submitted this for publication.

Background

One of the greatest needs for coronavirus disease 2019 (COVID-19), outside of a successful vaccination programme, is effective treatments. Ostensibly, the early response from the experimental medicine community to the first wave was robust, with more than 1,970 clinical trials planned, recruiting, or completed, at the time of undertaking this project in July 2020;[475] this has risen to 2478 at the time of writing in January 2021. This enabled enrolment of patients to trials of drugs with known safety profiles – including lopinavir,[476] remdesivir,[477-479] hydroxychloroquine[480,481] and tocilizumab[482,483]. The Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial has been particularly effective in the UK, having demonstrated a 12.1% absolute risk reduction in mortality among ventilated patients treated with dexamethasone,[484] and a further reduction in mortality afforded by treatment with tocilizumab (preprint result at [485]). It has additionally provided conclusive negative results, with no clinical benefits of lopinavir,[486] hydroxychloroquine[487] and azithromycin.[488]

However, while many of these trials have been pragmatic in terms of selection criteria, the proportion of hospitalised COVID-19 patients being recruited to clinical trials is lower than might have been anticipated; the authors of the RECOVERY trial estimated a 10% recruitment rate in the UK in April 2020.[489] Meanwhile, in areas where public health measures limited viral transmission, trials terminated early on account of under recruitment.[490,491] With mounting concern about an ensuing second wave of infection in the summer of 2020,[492,493] it was increasingly important to learn lessons from the first, and consider the number, size, and design of clinical trials that could feasibly be completed.

We hypothesised that the proliferation of SARS-CoV-2 interventional studies during the pandemic and under recognised barriers to recruitment of COVID-19 patients led to unachievable recruitment targets in England. I used data from clinical trial registry databases to quantify recruitment targets and concurrently studied recruitment rates, including reasons for exclusion, across 5 centres enrolling patients at the peak of the first wave of the pandemic. In conjunction with publicly available data from the UK

government, I considered the differences between the trials community's aspirations and delivery, and how this might inform the strategy for a second wave.

Methods

Establishing recruitment targets for registered trials during first wave

COVID-19 clinical studies registered on clinicaltrials.gov or the International Standard Randomized Controlled Trial Number (ISRCTN) databases were identified, and study data downloaded on July 12, 2020. Data for trials based in England, multinational trials with centres in England, and global trials were extracted in turn. Cross-registered studies were identified and accounted for once in the analysis. A manual review determined whether sponsors were academic, non-academic or mixed. Trials were excluded if they were labelled as terminated, withdrawn or suspended. Data for interventional trials examining treatment and prevention were documented, but only trials of COVID-19 treatments were used in the analysis. Analyses were performed using RStudio Version 1.2.5042.

Observational study of recruitment of hospitalised patients

We performed a prospective observational study of 2,082 consecutive patients with SARS-CoV-2 infection at 5 hospitals affiliated to the NIHR-Translational Research Collaboration with representation from secondary and tertiary centres: Cambridge University Hospitals NHS Foundation Trust (CUHFT), Cambridge; Imperial College Healthcare, University College Hospital and King's College Hospitals, London; and University Hospital of North Tees, Middlesbrough. Subjects were admitted and eligibility assessed for: RECOVERY (ISRCTN50189673), C19-ACS (NCT04333407) or SIMPLE (NCT04292730/NCT04292899). CUHFT local R&D approval was undertaken.

Demographic and clinical data were collected by contemporaneous review of potential participants' case notes. A categorical approach subdivided primary reasons subjects were not enrolled into: (a) clinical grounds (the screening or treating physician judgement that comorbidity or other reason for admission was

more critical to patient outcome than COVID-19), (b) being medically fit for discharge, (c) receiving end of life care, (d) lack of capacity, (e) patient refusal, (f) interactions between pre-existing medications and trial drugs, or (g) being on mechanical ventilation. Though already being on mechanical intervention was not an exclusion criterion for RECOVERY, patients categorised as excluded on these grounds were ineligible on account of competing, intensive care-based, studies.

Establishing feasible recruitment for registered trials during first wave

Using publicly available UK government data of the numbers of patients with COVID-19 admitted to English hospitals during the first wave between March 17 and August 5, 2020,[494] and the recruitment rate (with 95% confidence interval (CI) for one sample proportion with continuity correction) from the aforementioned observational study, we estimated a maximum bound for the accumulated feasible recruitment during that time. Simultaneously, we used the estimated cumulative number of infected cases in England by 12 July provided by MRC Biostatistics Unit at the University of Cambridge[495] to calculate an approximate hospitalisation rate in England among COVID-19 infections. We based our estimates on data from centres in England as the infection rate estimates were more reliable, hospitalisation criteria were different in Wales,[494] and the 5 hospitals included in this study are all from England.

Results

Establishing recruitment targets to registered trials during first wave

Clinical trial registry data were downloaded on July 12, 2020; 28 interventional studies were included in our analysis of those registered in England. 22 (78%) were academically sponsored, 5 (18%) were non-academically sponsored and 1 (4%) was mixed. The first registration date of a COVID-19 treatment trial in England was March 22; the earliest registered start date was March 12. Analysis of recruitment targets for each trial revealed that 46,154 participants would have been required to complete recruitment to all studies in England: 17,012 people required for trials of prophylactic drugs to prevent COVID-19, while 29,142 were needed for those

treating established COVID-19 (Table 7.1). The median (IQR) treatment trial recruitment target was 195 (50-793).

	Number of Trials	Number of Participants
Global Trials		
Prevention	172	260,446
Treatment	935	306,426
Total	1,107	566,872
UK Multi-National and National Trials		
Prevention	11	97,272
Treatment	38	44,362
Total	49	141,634
England Trials		
Prevention	8	17,012
Treatment	20	29,142
Total	28	46,154

Table 7.1. Summary of number of trials and required numbers of participants by July 12, 2020.

By contrast, the global situation was such that 1,107 registered interventional trials were ongoing or completed, requiring 566,872 patients to be randomised to allow their completion; 306,426 of these were needed for trials of COVID-19 treatments (Figure 7.1A and 7.1B). These trials were geographically clustered in China, North America and Europe (Figure 7.1C).

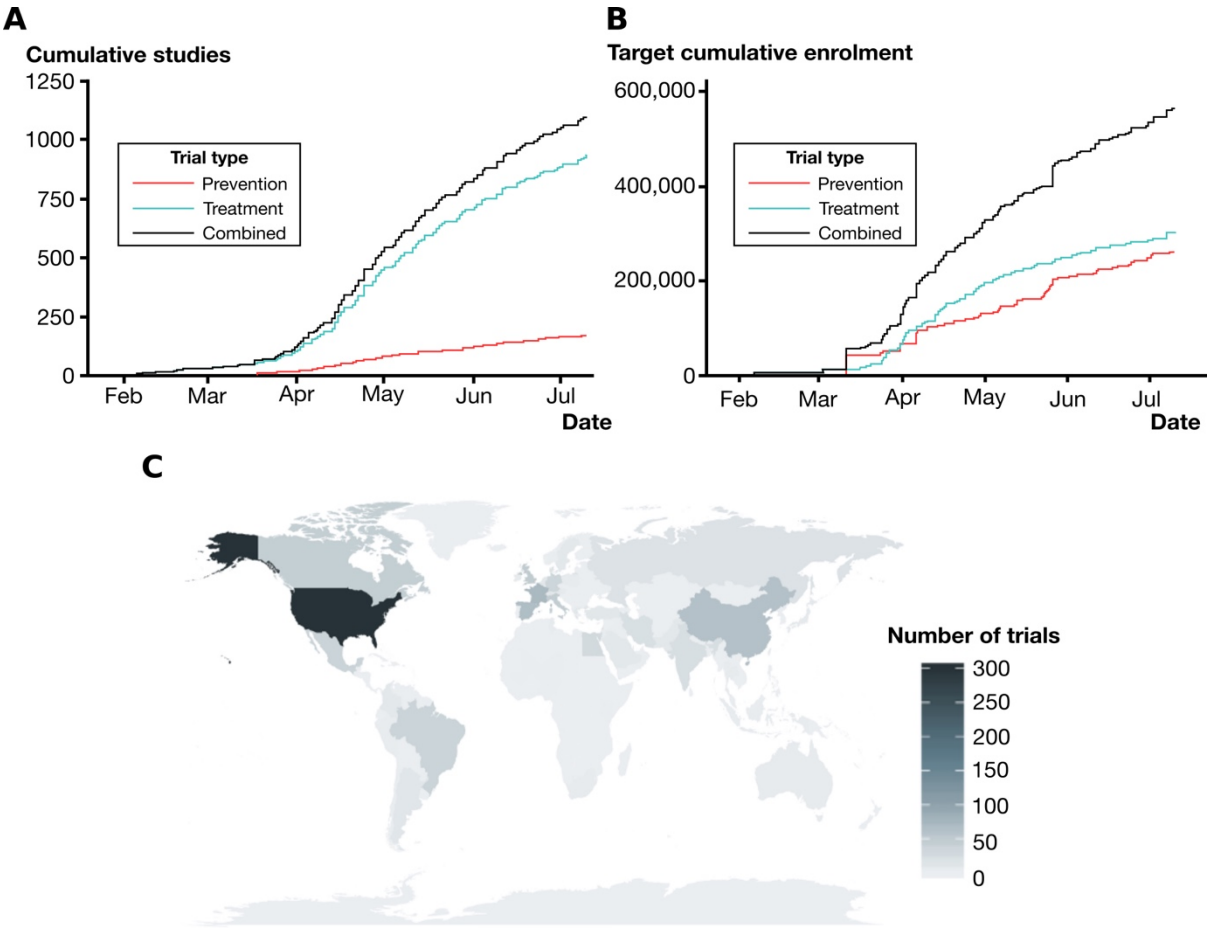

Figure 7.1. The proliferation of global clinical trials in response to COVID-19. A: cumulative number of enrolling studies registered with clinicaltrials.gov or ISRCTN until Jul 12, 2020, subdivided by those testing drugs for COVID-19 treatment and prevention. B*:* cumulative number of participants required to meet recruitment targets for registered clinical trials. C: geographical distribution of COVID-19 clinical trials.

Observational study of recruitment of hospitalised patients

From March 27 to May 22, 2020 a total of 2,082 consecutive patients were included across the 5 sites (Table 7.2). Age and sex data were available for 1,971 patients: the median (IQR) age was 71 (58-82) and 56.2% were male. Across the four trials, 430 (20.7%; 95% CI 18.95%, 22.47%) proceeded to randomisation.

Of the remaining 1,652 patients, 82 (3.9%) declined participation, 363 (17.4%) were medically fit for discharge, 153 (7.3%) were receiving end of life care and 106 (5.1%) were mechanically ventilated at the time of screening. In 699 (33.6%) patients, the

screening or treating physician determined that the potential participant should not be enrolled on account of clinical grounds or trial exclusion criteria.

	RECOVERY				COMBINATION *	C19-ACS	SIMPLE	**Total**
Total screened per centre	281	83	415	Total (779)	445	784	74	2,082
Number recruited (%)	35 (12.5)	16 (19.3)	185 (44.6)	236 (30.3)	124 (27.9)	56 (7.1)	14 (18.9)	**430 (20.7)**
Refused participation (%)	10 (3.6)	19 (22.9)	16 (3.9)	45 (5.8)	8 (1.8)	29 (3.7)	0 (0.0)	82 (3.9)
Clinical grounds/trial exclusion criteria(%)	83 (29.5)	15 (18.1)	40 (9.6)	138 (17.7)	167 (37.5)	365 (46.6)	29 (39.2)	699 (33.6)
Lacked capacity (%)	22 (7.8)	0 (0.0)	1 (0.2)	23 (3.0)	16 (3.6)	98 (12.5)	0 (0.0)	137 (6.6)
Mechanical ventilation (%)	37 (13.2)	7 (8.4)	0 (0.0)	44 (5.6)	7 (1.6)	48 (6.1)	7 (9.5)	106 (5.1)
Drug interactions (%)	12 (4.3)	2 (2.4)	0 (0.0)	14 (1.8)	2 (0.4)	1 (0.1)	0 (0.0)	17 (0.8)
Medically fit for discharge (%)	55 (19.6)	14 (16.9)	77 (18.6)	146 (18.7)	65 (14.6)	136 (17.3)	16 (21.6)	363 (17.4)
Palliative care (%)	19 (6.8)	7 (8.4)	61 (14.7)	87 (11.2)	8 (1.8)	51 (6.5)	7 (9.5)	153 (7.3)
Not approached or considered (%)	8 (2.8)	3 (3.6)	35 (8.4)	46 (5.9)	48 (10.8)	0 (0.0)	1 (1.4)	95 (4.6)
Total not recruited (%)	246 (87.5)	67 (80.7)	230 (55.4)	543 (69.7)	321 (72.1)	728 (92.9)	60 (81.1)	**1,652 (79.3)**

Table 7.2. Screening data for 2,082 consecutive patients with laboratory-confirmed SARS-CoV-2 admitted to one of 5 centres. *centre screened concurrently to both RECOVERY and SIMPLE: moderate and severe trials

Establishing feasible recruitment for registered trials during first wave

By combining these observed recruitment rates with publicly reported hospitalisation data (between March 17, and July 12, 2020), we estimated a maximum upper bound for the accumulated feasible recruitment for registered trials of COVID-19 treatments in England during the first wave (Figure 7.2).

The estimated number of cumulative infected cases by 12 July reported by MRC Biostatistics Unit was 4.67 million with a 95% credible interval (3.76, 6.04).

Combined with the number of cumulative admitted patients in England by 12 July from government data (i.e. 111,037 hospital admissions), this gave an approximate hospitalisation rate 2.38% (1.84%, 2.95%) in England during the first wave.

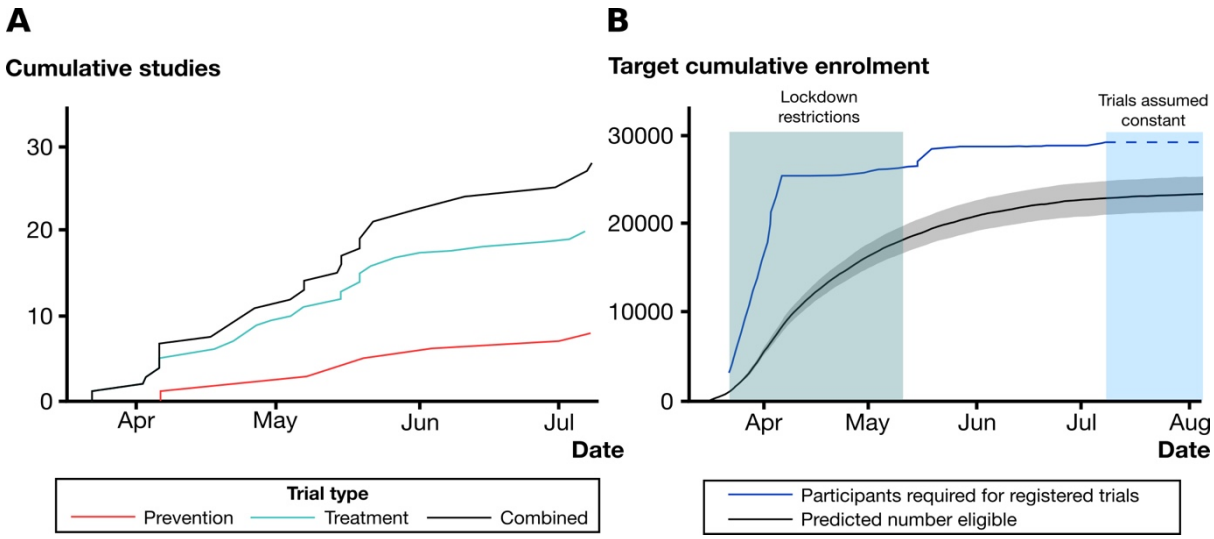

Figure 7.2. Feasibility of achieving target recruitment in England for COVID-19 interventional studies. A: cumulative number of enrolling studies in England registered with clinicaltrials.gov or ISRCTN until July 12, 2020, subdivided by those testing drugs for COVID-19 treatment and prevention. B: cumulative number of participants required to meet recruitment targets for registered COVID-19 treatment trials until July 12, 2020, and predicted number of patients whom would have been eligible for randomisation (grey shaded area represents point-wise 95% confidence band for the predictive cumulative number of eligible patients using the lower and upper value of 95% confidence interval for the recruitment rate estimate with continuity correction). The reduction in the infection rate in England meant that the recruitment target at July 12 was unlikely to be reached without a second wave; further illustrated by extending hospitalisation data to August 5, 2020.

This analysis indicated that by July 12th, 6,158 patients might still be needed to meet the total recruitment targets for currently recruiting clinical trials. If considering uncertainty in recruitment rate estimate reflected by 95% CI (18.95%, 22.47%), 4,192-8,100 patients might be required to meet recruitment target. Assuming the recruitment rate 20.7%, this implies that 29,749 hospitalised patients would need to

be screened for these trials to complete recruitment. With the approximate hospitalisation rate 2.38% in England as observed in the first wave, this would require a further 1.249 million patients to be infected.

With the daily infection rate for UK estimated to be 3,310 (95% credible interval of 2440, 4460) on 12 July,[495] it was highly unlikely such a large number of hospitalisations would occur without an increase in the infection numbers as seen in the second wave. Indeed, incorporating hospitalisation data to August 5, 2020, showed minimal progress toward the recruitment target, even assuming no new trials were approved after July 12, 2020 (Figure 7.2B).

Discussion

This study found that the proliferation of clinical trials[475] in response to the first wave of the COVID-19 pandemic in England required 29,142 participants to complete enrolment to those registered with a trials database. Globally, 306,426 participants were required to meet recruitment targets for trials of treatments of COVID-19. Meanwhile, in this multicentre prospective observational cohort study of patients admitted to hospital with laboratory-confirmed COVID-19, 79.3% of potential participants were not recruited to a clinical trial; the reasons for excluding patients were varied and clarify the challenges faced in both general hospitals and well-resourced centres experienced in experimental medicine. This experience was consistent with the general literature on clinical trial recruitment where many factors have been posited to contribute to heterogeneity of recruitment.[496] With 111,037 people hospitalised in England between March 17 and July 12, 2020, the net recruitment rate suggested that 22,985 (21,042-24,950 if taking into account uncertainty in recruitment rate estimate by random errors) would have been potentially suitable for selection in the first wave. However, this is clearly an overestimate, given that it would require each of these individuals to be hospitalised in geographical locations where medical centres were undertaking these trials. We concluded that unless there were a second wave it would be highly unlikely that the total recruitment target will be met in any reasonable timeframe.

Strengths of my study were that the analyses of registry and population databases utilised the largest and most robust data available. Meanwhile, my observational study applied a large cohort size, prospective data acquisition, and recorded detailed reasons for excluding patients. By using both secondary and tertiary care centres, we concluded our results were generalisable to other hospitals in the UK. Also, by following studies with minimal selection criteria, particularly in the RECOVERY trial, we reduced the chance of underestimating trial recruitment.

The study did have limitations, however. First, the predictions were based on registry data for studies based in England alone; we did not include the numbers of participants required to be recruited into multinational trials in which the English centres were involved. The result is that we likely underestimated the trial recruitment target for England and, by extension, the gap between this and the number of participants available. Second, although we used hospitalisation data from 17 March 2020, as this was the time the UK government commenced public reporting of COVID-19 admissions, all trials included in the registry analysis were not recruiting at that stage; the earliest start date for a trial registered in England was March 12, 2020, but the last trial start date was not until July 7, 2020. In this sense, using cumulative number of admitted patients in our prediction was optimistic. Third, we only included the two registry datasets in most widespread use, and so may have further underestimated the number of studies and participants required. Fourth, the 95% CI for recruitment rate estimate only reflects the uncertainty due to random errors in the data, it does not consider the uncertainty due to unrepresentativeness of data from the 5 hospital centres in our study. Finally, although I illustrated the scale of trial recruitment required globally, the populations tested may not be representative of, or translatable to, international cohorts.

This study was the first to characterise the suitability and barriers for trial enrolment for a complete cohort of hospitalised patients with COVID-19. Results of trials published to the point of publication had conveyed a different message: interventional studies of lopinavir and remdesivir, for example, had recruitment rates

ranging from 55.7%-96.0%.[476-478] This difference is most likely explained by the different denominators used in our calculations: the CONSORT diagrams in clinical trials are unlikely to include every single patient hospitalised with a positive test. Instead, our results align with or exceed other centres, such as the 10% recruitment rate to RECOVERY.[489] During the 2013-16 Ebola Virus Disease (EVD) epidemic in west Africa, most clinical trials during that crisis either started too late to enrol sufficient case numbers or were simply unable to reach their recruitment targets.[497] This study showed that trials in England started recruiting relatively quickly, however many were highly unlikely to recruit on time; we concluded that starting early is important but not enough to ensure recruitment targets are met. Finally, it is notable that our calculated hospitalisation rate of 2.38% is lower than that observed in Wuhan,[498] which if applied to the UK age structure,[499] is equivalent to approximately 5.8%.

The disparity between the realistic recruitment rates and high requirements we reported led us to conclude that the scientific community should be increasingly selective in the number, size and design of clinical trials deployed in the COVID-19 pandemic; our findings had meaning for those planning single trials, and those strategizing the national response. Potential solutions include practical changes to trial design, for instance capturing patients earlier in their disease path, and adopting dynamic and adaptive trial designs.[500] Yet, such measures seemed unlikely to bridge the estimated large recruitment gap. Instead, we concluded it may be necessary for healthcare authorities and policy makers to foster more academic cooperation to prioritise compounds, prevent duplication and, perhaps more radically, perform real-time meta-analyses of ongoing trials of the same therapies and provide stop/go recommendations across trials to rationalise treatment and prevent multiple studies delaying reporting.[501] Indeed, proposals had been forthcoming for mechanisms by which data from different trials might be shared and analysed in a robust and scientifically meaningful way.[502] These conclusions are not dissimilar to reflections from the Ebola pandemic, when there was a strong call for strengthening and coordinating research efforts in response to outbreaks of emerging infectious diseases.[503,504] For planning future trials and deriving realistic recruitment targets,

real-time tracking of the pandemic, as data accumulate over time, is essential to plan research in response of an emerging epidemics outbreak. The Medical Research Council (MRC) Biostatistics Unit regularly report and forecast COVID-19 infections and deaths.[495] This information feeds directly to SAGE sub-group, Scientific Pandemic Influenza sub-group on Modelling (SPI-M) and to regional PHE teams. This same data could be used to establish realistic recruitment trends to inform, monitor and coordinate research efforts both for treatment and prevention trials.

Our primary conclusion was that clinical trialists and healthcare authorities must consider the recruitment challenges when determining the feasibility of clinical trials in a second wave, while urgently rationalising those currently active. At the time of publishing this study, it remained unclear how relaxing of non-pharmacological interventions would affect transmission rates, and therefore the achievability of remaining recruitment to these trials. Of course, the ensuing waves of COVID-19 led to markedly increased hospitalisation rates. Applying the same methodology with hospitalisation and trial registry data up to Feb 01, 2021 (Figure 7.3), it is possible to see how the magnitude of the subsequent waves in England led to large numbers of potential participants, perhaps sufficient to meet recruitment demands. However, it remains noteworthy that the most successful studies in this pandemic have been those undertaken by collaborative initiatives. Special attention must be given to these successful studies, so that trial policies can be developed to prepare for future crises.

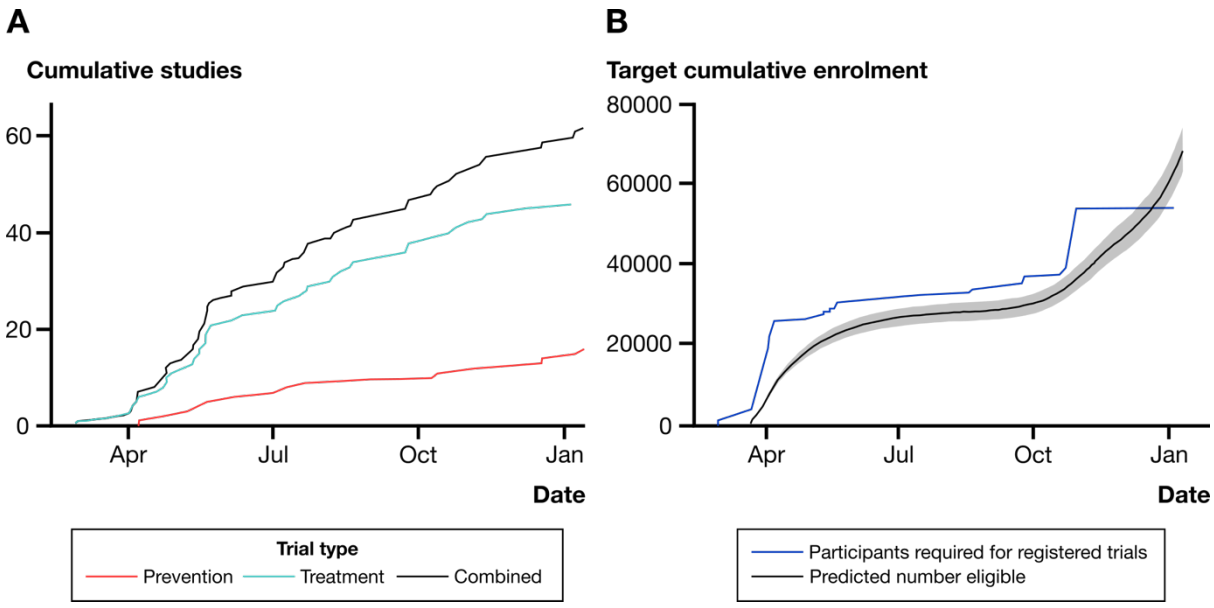

Figure 7.3. How subsequent waves of COVID-19 have affected the gap between target and predicted clinical trial recruitment in England. A: cumulative number of enrolling studies in England registered with clinicaltrials.gov or ISRCTN until Feb 01, 2021, subdivided by those testing drugs for COVID-19 treatment and prevention. B: cumulative number of participants required to meet recruitment targets for registered COVID-19 treatment trials until Feb 01, 2021, and predicted number of patients who would have been eligible for randomisation.

Chapter 8: Conclusions and future plans

Currently available disease modifying treatments for multiple sclerosis are, predominantly, immune modulatory and do not directly promote repair. However, the treatment of MS has arrived at a new and exciting juncture in which a myriad of medications, both novel and repurposed, are being evaluated on their ability to elicit repair of myelin, and thereby reduce disability accrual. Our work with bexarotene has, for the first time with converging evidence from electrophysiology and MRI, shown that a small-molecule drug can therapeutically enhance remyelination in people with relapsing remitting multiple sclerosis. While the side effect profile of bexarotene will preclude its further testing in people with MS, CCMR One has highlighted a druggable pathway that might be more selectively targeted to achieve its therapeutic effect without the breadth of adverse effects we observed. It has also emphasised the sensitivity of electrophysiological assessments of the visual pathway to detect remyelination, and it has highlighted regional variations in the responsiveness of MS lesions to a putative remyelinating drug. An additional learning, elaborated in this thesis, has been the reduced responsiveness of older individuals to bexarotene. This final point is particularly pertinent given recent research has identified interventions with the potential to reverse the hallmarks of ageing in oligodendrocyte progenitor cells, by, for example, rejuvenating drugs like metformin and niacin. These lessons have featured strongly in our approach to CCMR Two, in which our therapeutic strategy is to combine a pro-differentiation drug (clemastine) alongside a rejuvenating drug (metformin), with the implementation of a trial design that we believe will be able to detect the structural and functional consequences of remyelination in a, comparatively small, single-centre study.

Of course, there exist several unresolved questions and potential limitations with such a therapeutic strategy. In particular, it remains unclear whether remyelination failure can be adequately resolved in humans by reversing the block to OPC differentiation, and there is uncertainty around what amount of remyelination would be clinically meaningful. Inevitably, clarification will also be needed on how a clinician should deploy a remyelinating drug, once a safe and efficacious one is identified. But

ultimately, the pathological processes that drive the accrual of disability in progressive forms of MS are multifaceted, and an approach outside of exclusively enhancing remyelination – for example one that targets remyelination alongside other mechanisms – may emerge. Optimism about such a method was exemplified during my work with the treatment selection group in which 2 of the 3 repurposed drugs chosen by the panel (metformin and niacin) were primarily remyelinating, but had additional effects on other targets thought relevant to the pathobiology of progression. Seeing these drugs evaluated in the MS Society's new phase of drug trials in progressive MS, with a range of outcome measures sensitive to both remyelination and neurodegeneration, will be one of the most exciting events in MS clinical research over the coming years.

It has been a disappointment that the COVID-19 pandemic significantly limited my clinical research, in particular by preventing any recruitment of patients to observational clinical studies (which, being within level 3 of the NIHR recovery strategy, have not been permitted to recruit participants in Cambridge since March 2020). I am, however, pleased to have been able to work to contribute to the successes of the RECOVERY trial, and to have been able to lead an important research project to document the barriers to trial recruitment during the first wave of the pandemic. I am additionally thankful to have been still able to conduct my CCMR One recall study, which, even with the limited time available, highlighted the reproducibility, sensitivity to change, and responsiveness to treatment of the VEP, with durable improvements evident more than 2 years after treatment with bexarotene.

Electrophysiology will undoubtedly continue to play an important role in phase II remyelination trials and should support the realisation of the therapeutic promise of a remyelinating drug. The full-field visual evoked potential has, as elaborated in this thesis, emerged as the most sensitive outcome measure across the remyelination trials to date. The additional sensitivity and specificity of multifocal VEP to capture changes in small regions of optic nerve demyelination, has promoted its inclusion as an outcome for both acute and chronic remyelination trials. Meanwhile, an alternative

technique, that has already proven useful in quantifying pathology in other neurological diseases, is the measurement of saccadic reaction times. While prolonged saccadic latencies can arise from multiple pathologies, and so lack the pathological specificity of the VEP, they remain stable over time for individuals so that sequential changes can provide a sensitive method for quantifying influences on neural mechanisms, including neuroprotection. The constraints of the COVID-19 pandemic have prevented me from conducting the complete assessment of these saccadic parameters in people with MS that I had intended, but I was able to gain pilot data between November 2019 and March 2020 from healthy controls and people living with MS (Appendix 2).

Given the frequent involvement of the visual pathway in MS, and the histopathologic similarities between optic nerve damage and MS lesions within the brain and spinal cord, I plan to continue to embrace visual pathway outcomes for remyelination in post-doctoral research. This aligns with recent investment in the development of an academic vision laboratory in the department of clinical neurosciences at Cambridge; a collaboration between Professors Alasdair Coles and Patrick Yu-Wai-Man. This resource, coupled with the skillset I have acquired over the duration of the PhD, allows me to apply the aforementioned electrophysiological tests, as well as measures of visual function including acuity, colour vision, optical coherence tomography and Humphrey visual fields.

My future plans are the following research projects:

The Cambridge Centre for Myelin Repair trial number Two. Having led the development of this trial, secured ethical approval (21/EM/0120) and established the skillset required for measuring the endpoints, I plan to see this study through to its completion, while also building experience in running clinical trials, under the supervision of an experienced chief investigator. Additionally, I will recruit participants to the study of progressive remyelination in nerves by grading electrophysiological recovery (SPRINGER); allowing me to test whether long term

changes occur in response to metformin and clemastine, in the same way I showed with bexarotene (Chapter 4).

Clinical study of saccadometry in MS. Assessments of saccadic latency have been advocated as a way to monitor response to neuroprotective therapies in the field of MS;[391] just as they previously have in Parkinson's disease[505] and Huntington's disease,[506,507] among others.[508] There remains a significant amount of work to be done before this possibility might be realised: they would need to be shown – in a large cohort of people with MS – to be reliable, reproducible, and be responsive to change. I have a research protocol authorised by the London-Hampstead committee (19/LO/1284) to test this; I will recruit 30 participants from the progressive MS clinic, while saccadic parameters will additionally be tested in the 50 RRMS participants of the CCMR Two trial. Three protocols will be tested: a ("reflexive") step task, an antisaccade task, and the Wheeless task.[509] The rationale is to evaluate the different neuronal populations that contribute to both reflex and voluntary eye movements that can become disordered in multiple sclerosis (and might improve with remyelination and axonal protection).

Submission of a research proposal to measure full-field, and multi-focal, VEP in a sub study of the OCTOPUS trial. As documented in this thesis, VEP has emerged as the most sensitive way to measure remyelination. Given that two from the top three drugs recommended by the treatment selection group have a primarily remyelinating mechanism of action, OCTOPUS offers an unrivalled chance to test the remyelinating effect of these drugs using the infrastructure we have put in place in the Cambridge academic vision laboratory. We propose to recruit trial participants from centres in London and the East of England to travel to Cambridge for visits at baseline, and if delayed VEP latencies are demonstrated, they will return at month 12 and month 24.

To bring measurements of remyelination and axonal health to routine clinical practice. My conclusions in this thesis support previous research that has described high inter-subject and inter-lesional variability in remyelination capacity. One of the

new research aims of the Cambridge Centre for Myelin Repair, now in its fourth phase, is to elucidate the deterministic factors for remyelination failure in people with MS, in particular to assess the contribution of age, so that we might better understand how and when to deploy a remyelination strategy. We plan to achieve this by becoming the first MS centre to bring measures of remyelination and neuronal health to routine clinical practice. I will first collaborate with MRI physicists and radiologists in Cambridge to develop an MTR sequence on our NHS 3T GE scanner, before building and testing the analysis and processing pipeline, including registration and segmentation methods. This will allow automated lesion counting and quantification of cerebral volume, either in-house or through a third party such as Icometrix. 40 patients will then be recruited to a pilot trial from the Addenbrooke's DMT, paediatric (n=5) and progressive MS clinics. Participants will attend at baseline, month 6, and month 12; with the option for extra visits in the circumstance of an acute relapse. At each visit, participants will have an MRI, a full-field and multifocal VEP, LCLA and OCT performed. These pilot data will determine which assessments of myelination and axonal health will be taken into clinical practice as part of a well-powered study, and set the foundation of a research platform that will allow future researchers to assess the influence of lifestyle, age, stage of disease and treatments on remyelination, and to optimise therapeutic strategies to enhance neuroprotection.

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Appendix 1: Intermittent fasting as a way of promoting remyelination in patients living with multiple sclerosis:

A focus group to inform the design of a study of intermittent fasting in MS

In order to develop a dietary intervention involving fasting, that takes into account the needs of patients with MS, we held a focus group at the Addenbrooke's brain repair centre on Wednesday 25th July 2018. The intention was to engage with the public at an early stage of our trial's development through opening a dialogue with a body of people living with MS. The MS Society, which has a research network comprised of approximately 250 people with MS, supported us in this endeavour by recruiting a group of representative patients.

Attendees

Healthcare professionals: Alasdair Coles (Professor of Neuroimmunology), Nick Cunniffe (Neurology Registrar), Katie Carr (Academic nutritionist)
MS Society public engagement officer: Jenny Robertson
Participants: 6 people with RRMS, 1 person with PPMS, 2 people with SPMS, and 3 carers/friends. All were members of the MS Society research network.

Statement of objectives

In planning the afternoon, there were a few aims we had for our discussions:
1. To better understand how a diet involving intermittent fasting might positively and negatively impact on someone living with MS.
2. To gauge what sort of diet might be feasible and tolerable for the duration of a clinical trial.
3. To discuss the different ways in which we could encourage compliance.

4. To appreciate how significant a positive effect would have to be for someone to follow an intermittent fasting diet.
5. To discuss how one might pragmatically design a clinical trial of a dietary intervention in people with MS.

Outline of programme

Following a welcome and introduction from both the MS Society's representative and Prof Coles, a short presentation was given to outline the latest research which had led to the hypothesis that fasting in humans would enhance remyelination. The conversation was then opened to the group, with the following questions used to structure the discussions:

1. Has anyone tried any similar diets before? What were the positive and negative effects?
2. Do you think you could undertake a diet involving fasting?
3. For how long do you think you could sustain an intermittent fasting diet?
4. In the design of a trial, how would you encourage compliance?
5. What would be the magnitude of therapeutic effect would you want to gain to undertake such a diet?
6. In the design of the trial, how would you monitor participants?
7. Any other comments or insights?

Discussion points

The group started by detailing their own experiences of dieting while living with MS. One patient explained she had undertaken a diet limiting herself to 600 calories a day for a period of 5 weeks, with the intention of losing weight. She reported that she lost 20lbs with no adverse effects and that a particular help with compliance had been use of low calorie sachets, soups for example, which gave her defined amounts that she could eat. Another patient explained that she had trialled an intermittent fasting diet (5:2), but had to stop on account of developing headaches that limited her ability to work. However, she subsequently tried gradually increasing the degree of fasting over several weeks, which counteracted the problem. Both

explained that they had noted an improvement in their well-being and strength, though others in the group did raise the possibility that this might be the effect of their weight loss rather than an effect on their MS.

There followed a conversation about the literature and other diets that have previously been trialled. One patient volunteered to having tried the “fasting mimicking diet” reported by Choi et al. in 2016.[510] This involved one pre-fast day of 800kcal in brown rice, followed by 7 consecutive days of 200 calories a day, which was taken as a vegetable broth. He had initially tolerated this well, but became gradually more weak and fatigued towards the end of the fast. He explained that he lost weight but noticed no convincing improvement in his MS symptoms. He did however find he had much more free time without the daily need to prepare food.

Subsequently, there was a discussion around some of the barriers to fasting. Firstly, a couple of patients raised a concern that some disease modifying treatments have side effects that might make a complete fast for a whole day rather more difficult. For example, one patient emphasised the importance of taking her Tecfidera with food, which negated GI side effects. Also discussed were the social implications of fasting, and some expressed a wish that fasting days be flexible so that they could plan them into their week. Despite this, all in the group believed that a diet incorporating fasting was achievable. However, very few believed that a diet akin to that used in Franklin’s experiments (absolute fasting Monday, Wednesday and Friday) would be possible for a prolonged period. Far more preferable would be 2-3 non-consecutive days of a calorie-restricted diet (such as 500 calories on these days).

Such considerations fed into a discussion about the length of time such a diet could be trialled. All agreed that, for a diet with limited calories, 3 to 6 months would be very achievable. However, if it were a matter of fasting MWF, then 3 months was felt to be a limit. When the suggested time period approached 6 months, some explained that their compliance would be more likely to stray and that they would be happier if they were seeing an improvement, either in their physical health or in some objective marker of disease activity, their MRI for instance. However, all said that, if there were

an effect on the natural history of their MS, even years into the future, proven in a study such as the one proposed, they would be motivated to maintain a diet that involved intermittent fasting indefinitely.

There was less of a consensus when it came to the dialogue about how best to encourage compliance. Some of the group were confident that they would need no contact from the trial team in order to keep up a fasting diet. Similarly, some also felt no contact would be needed, but that they would benefit from having aids such as the defined amounts in sachets. A few participants thought that it would be a good idea for the trial team to keep in touch with patients, particularly towards the start of the trial period, by email or telephone calls. One idea that was mooted was to have an app where they could record their meals, which might be linked to investigators of the trial. The most agreement came from wanting to know that someone would be available to speak to if they needed while on the trial.

Katie, the research nutritionist, explained that an alternative to sachets would be for her to provide a series of low calorie meal recipes and low calorie snack ideas, which was well accepted by the group. Most agreed that this would allow more choice for the patients and so appeared to be the best option. She also explained how she, and a dietician, would monitor people on the diet.

One of the questions put to the group was, if we could quantify the positive effect of the diet, how large a benefit would they want before they would consider such a diet. All agreed that they would do this if there was a reduction in the rate of progression and wouldn't need to be told that this would stop or even reverse disability.

In terms of trial design, there were some very helpful suggestions from the group. It was put to the participants that, if you were allocated to a "control" diet in a study, would you continue with your normal diet or would you try fasting yourself? Several agreed that it would be hard to carry on as normal in such a scenario, knowing that others in the study might derive a positive effect. In meeting the need for a control population, some in the group thought that there would be patients from MS clinics

who would be willing to have the baseline and follow up investigations and opt out of the diet. Another suggestion was a crossover design, in which participants were enrolled, had a control diet for 6 months, and then underwent the diet for 6 months, with investigations at 0, 6, and 12 months. A final idea was to have a dose ranging study, with some on a fasting diet, some on a low calorie, and some on a control diet.

The next consideration was blinding and it was recognised that it would be very hard for patients and doctors to be blinded to whether the patient is on the trial diet or not; ideas such as high calorie sachets were quickly dismissed by the group. However, it was agreed that an objective measure, with blinded raters, such as with MRI or VEP, would be a sensible option and acceptable to those that might participate.

As we came to a conclusion, we discussed the bias that might be involved in the information gathered from this focus group. All had responded to an advert from the MS Society to take part in a “discussion regarding intermittent fasting to promote remyelination”. The group therefore described themselves as fairly self-selecting: they were all interested in the topic of diet in MS, as well as already being actively involved with research through the MS Society research network. The group did say they thought their outcomes were valid to the MS community at large, but noted that there may be some challenges, especially with compliance, with a more diverse cohort.

Results of questionnaire

In order to gain some more quantitative information from our participants we also asked the attendees to complete a short questionnaire. Of our group of 12, 11 completed the form, though 1 of these did so only partially. The mean age of respondents was 51 years and, in those living with MS, the mean length of time with this was 13 years. Average levels of alcohol intake were low with a mean of 6 units a week and only one person reported to an intake greater than their recommended weekly amount (42 units for one gentleman).

All respondents said that they would be willing to undertake a diet involving intermittent fasting. When asked how long they would be willing to try this, 100% reported they would be happy with at least 6 months. Questions on how might be best to monitor compliance showed no clear consensus: 70% preferred email contact with the trial team, while 30% preferred telephone calls. Only 2 respondents were happy to complete food diaries.

Finally, the hypothetical question, “if we could quantify the positive effect of intermittent fasting on the course of MS, how large would this need to be for you to follow the diet”, was asked. Of the 9 respondents that completed this question, all said they would follow the diet even if it only slowed progression marginally by less than 20%.

Summary

- The majority of participants had already trialled diets involving fasting, motivated by weight loss or improvement in MS symptoms.
- Generally, these were well tolerated and patients felt better for doing so, though some noticed headaches and, in cases of more extreme fasting, increased fatigue.
- Everyone in the group thought they could change their diet to one involving intermittent fasting. However, while a couple thought 3 non-consecutive days of fasting was possible, most did not, and all agreed that any more than 3 months on such a diet would be problematic.
- All were open to 2-3 days of fasting with low calorie (<500) meal options on the fasting days for a period of at least 6 months.
- In order to encourage compliance, it was suggested the fasting days be flexible to allow for social occasions and, while some would find low calorie meal sachets a help, most preferred the option of low calorie meal and snack recipes from a nutrition or dietician.

- In the trial design, there was concern that a control population would not observe their normal diet, and so a crossover design or recruiting MS patients whom do not want to undertake a diet were suggested as potential options.
- Blinding of patients was felt to be impractical, but participants would be comfortable if there were an objective measure such as MRI with a blinded rater.
- The conclusions of the focus group were believed to be translatable to the wider MS population, but there was agreement that the assembled group were a selective population interested in both MS research and dietary interventions.

Appendix 2: Oculometry in multiple sclerosis

Pilot saccadometry data from people living with MS

Background

Each saccade is the result of a decision on where to look, and the time needed to make these decisions can provide precise information about the underlying neural mechanisms of decision, distributed amongst many structures in the brain, both cortical and sub-cortical.[511,512] Saccadic latencies are typically delayed by around 100-130 ms more than would be predicted from considering the shortest pathway for a visually evoked saccade (from the retina through the brainstem, to the extraocular muscles). This is known as oculomotor procrastination: the cortex tonically inhibits the collicular mechanisms via the basal ganglia, only releasing this inhibition briefly and locally after the decision process is complete.[513]

Although distributions of saccadic latency have much inter-subject variability, they remain relatively stable over time for the individual so that sequential changes can provide a sensitive method for quantifying influences on neural mechanisms responsible for their generation, such as disease or therapeutic intervention. Saccadic reaction times are known to be abnormal in a very wide variety of neurological disorders,[506,514-519] they correlate with grey matter volumes in Parkinson's disease,[520] and saccadic paradigms are being increasingly used to help give insight into disordered neural processes. Meanwhile, in the field of MS, one group has published several studies from the same cohort of 25 people with multiple sclerosis (MS).[392-395] They reported deficits in complex decision making, such as prolonged latency and increased proportion of errors (prosaccades) in the antisaccade task.[394] Because these measurements are so easy to make, they hold promise for being useful biomarkers of disease progression and treatment, particularly as outcome measures in clinical trials.[391] I therefore additionally sought to study saccadic latency distributions in people with MS, and describe how they vary with disability and disease duration.

Methods

In this project, I sought to compare distributions of reaction times in saccadic tasks of varying complexity between MS patients with both cross sectional, and longitudinal analyses. A research ethics application was authorised by the London-Hampstead committee in August 2019 (19/LO/1284).

Equipment and protocols

I used a head-mounted saccadometer plus (Ober consulting), which uses binocular infrared scleral reflectance to measure eye movements in response to the presentation of high contrast horizontal targets from three low-power lasers that project red 13 cd m^{-2} dots subtending 10° on a flat background. This device has FDA approval for use in the clinical setting, is non-invasive, and does not require restraint. Two protocols were tested (Figure A2.1): a ("reflexive") step task and an antisaccade task.[521] The rationale for this was to evaluate the different neuronal populations that contribute to both reflex and voluntary eye movements that can become disordered in multiple sclerosis (and might improve with remyelination).[513] Further, as seen below, the sending of a "stop" signal to cancel a movement is more rapid process than required for the step task; I hypothesised that this might be more sensitive to changes with remyelination.

Analysis

Latency data were downloaded onto a computer running LatencyMeter®. This software automatically removes abnormal eye movements. These include saccades in the wrong direction (in the step task), those with an abnormal profiles, those falling outside the amplitude range 5–15°, or saccades outside the latency range 50–600 ms (arbitrary limits chosen to include express or other early saccades but exclude saccades with prolonged latency due to inattention). Each is also checked manually.

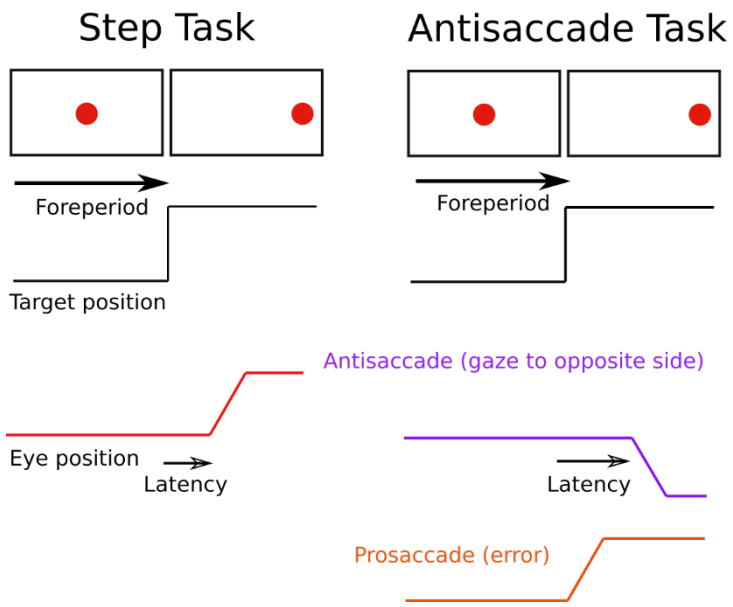

Figure A2.1. The step and antisaccade tasks. In the step task paradigm, after a random period of between 0.5 - 1.0 seconds, the central target is extinguished and, simultaneously, either the left or right target presented. The device measures the latency of the resulting saccade (time between presentation of the target and eyes starting to move). The antisaccade paradigm involves the same sequence, but the subject is instructed to look in the opposite direction to the stimulus; thus one can respond with either an antisaccade or make an error (a prosaccade).

The resultant saccadic latency distributions are skewed with a tail towards longer latencies. However, if instead of latency, one plots the reciprocal of this reaction time (a measure of the rate of decision), a normal distribution is formed. This reflects the underlying biology of the decision making process, which is generally agreed to involve a rise-to-threshold decision signal; the brain accumulates sensory information toward a threshold level, at which point a certain action is selected.[509,522] If reciprocal latencies are plotted cumulatively on a probit scale (forming a reciprobit plot), then a straight line is obtained and the results can be explained using an established model of neural decision: the LATER model (linear approach to threshold with ergodic rate; Figure A2.2).[509,522]

The LATER model is particularly powerful as it provides a more sophisticated measure of the neural decision-making process than basic behavioural measures,

like the mean latency of response, or proportion of errors made. It is also supported by neurophysiological measurements from movement cells in the frontal eye fields in monkeys,[523] and neuronal responses in the parietal cortex.[524] As a result, this approach has greater potential to support clinical trials than simply recording crude metrics like reaction time in isolation.[509]

Using the SPIC programme, I generated reciprobit plots for each participant and, using a Kolmogorov-Smirnov statistical test, obtained the best fit for the data and defined the distribution in terms of LATER parameters: the inverse median latency (μ)-essentially a measure of "promptness", and the variance (σ^2).

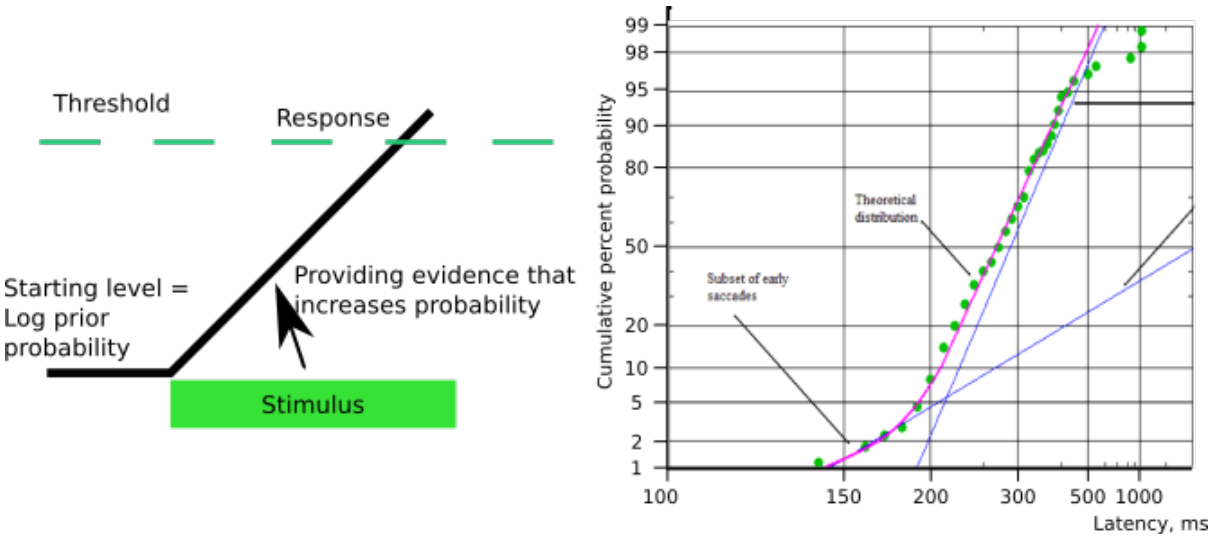

Figure A2.2. The LATER model. *Left*: The decision signal rises from a starting level, S_o, until it reaches a threshold criterion, S_T, at which a response is initiated. The rate of rise (arrow) varies randomly from trial to trial around a mean, μ. In the real world, competing LATER units race against each other, and the first to reach threshold initiates the response. *Right*: the reciprobit plot. When cumulative probability, on a probit scale, is plotted against inverse latency a straight line is formed, representing the normal distribution. This can be quantified in terms of its median, μ, its intercept with infinity, σ, and, if present, a population of "early" saccades can be described by the term σ_E.

Results

While the constraints of the COVID-19 pandemic have prevented me from conducting a complete assessment of these saccadic parameters in people with MS, I have been able to use the saccadometer to gain pilot data from seven healthy controls and seven people living with MS, between November 28, 2019 and February 27, 2020, administering the protocols specified in the methods. Saccadic distributions in each instance were used to generate reciprobit plots and obtain the best fit in terms of LATER parameters: inverse median latency (μ), variance (σ^2) and, if present, σ_E of the early distribution. Representative data for a single subject are shown in Figure A2.3.

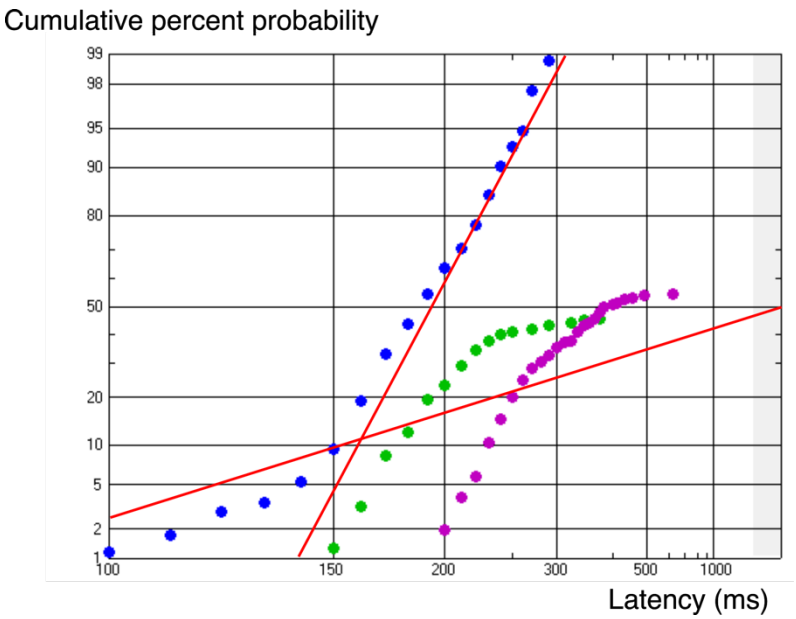

Figure A2.3. Representative results from one MS subject. A cumulative histogram, using a probit scale, is plotted against inverse latency – a normal distribution forms a straight line, which is quantified by median latency (μ) and its variance (σ^2). In this case distributions from the step task (median latency 190 ms, μ = 5.24; blue), and the antisaccade task (latency 267 ms, purple, with 45% prosaccadic error rate, green) are shown; an additional population of early saccades were present in this subject (σ_E).

As expected, in the step task, there was a statistically significant difference (p = 0.015) between the means of the median latencies of saccadic reaction times of people with MS (188.0 ms) versus the healthy controls (151.3 ms). Similarly, when these saccadic distributions were modelled using LATER, μ (inverse median latency or "promptness") was smaller in those with MS, compared to healthy controls (between group difference -1.39, p = 0.024, Figure A2.4A and B). σ, a measure of variability, was no different between the two groups (1.2 vs 1.3, p = 0.473), though 3 people with MS had a significant early population of saccades versus none of the healthy controls.

Antisaccades were assessed for the same participants and the results summarised in Figure A2.4C and D. Visually, this appears to demonstrate a trend for antisaccades to occur at greater latency in people with RRMS than those of healthy controls, but the means of these were not statistically significant from one another (311.6 versus 271 ms for MS participants and healthy controls, respectively, p = 0.261). While 2 people with MS had a large proportion of prosaccadic errors (Figure A2.4D), there was no statistical difference between the two groups.

Discussion

The main step I have been able to take has been to become familiar with the equipment, and to learn how to administer and analyse saccadic paradigms using the LATER model. The pilot data presented in this appendix, while only a small sample due to the study being halted at the start of the COVID-19 pandemic, does highlight prolonged saccadic latencies in people living with MS in both the step and antisaccade task, which is broadly in line with the only other study in this patient group to date.[391-395] A significant difference with previous work has been that I have not replicated the observation that people with MS have an increased proportion of errors (prosaccades) in the antisaccade task.[394]

Assessments of saccadic latency have been advocated as a way to monitor the response to neuroprotective therapies in the field of MS,[391] Parkinson's disease,[505]

Huntington's disease,[506,507] and others.[508] The hope behind this continuing line of research, is that changes in saccadic latency will vary with remyelination in a clinical trial, and potentially be more sensitive than established measures such as VEP. With tests of saccadic latency being introduced into the CCMR Two trial, there will be ample opportunity to evaluate these points in the setting of post-doctoral research.

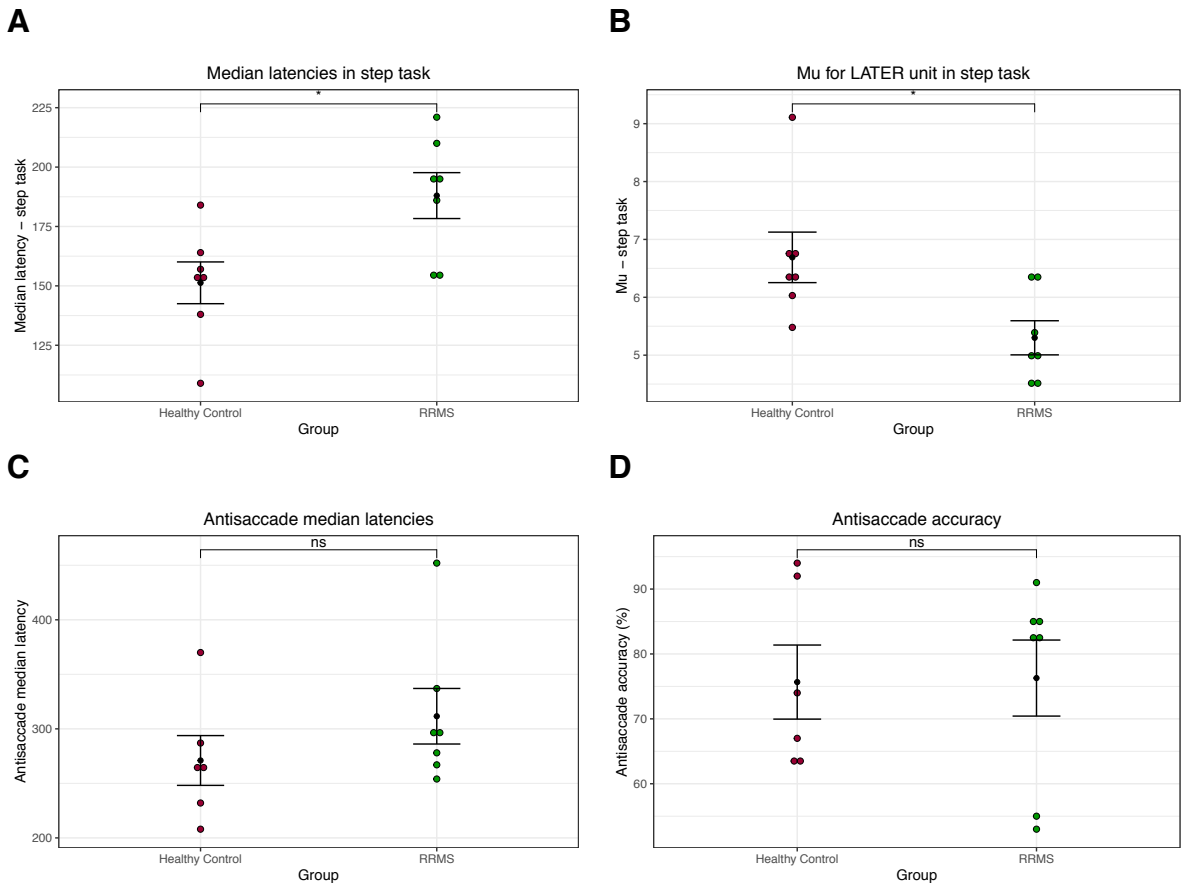

Figure A2.4. Between group differences in performance at the step and antisaccade tasks. A: the median latencies (in ms) for healthy controls and participants with relapsing remitting MS (RRMS) performing the step task. B: the inverse median latency, μ, of the LATER unit fitted to the latency distribution (μ is a measure of promptness, and higher values infer faster reaction times). C: the median latencies (in ms) for antisaccades in the same groups of participants. D: the proportion of correct responses (expressed as a percentage) during the antisaccade task (higher values mean fewer prosaccadic errors). Bars are standard errors around the group mean. *p<0.05.