Title page

Safety and efficacy of bexarotene in people with relapsing-remitting multiple sclerosis (CCMR One): a randomised phase 2a two-centre placebo-controlled trial

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Abstract

Background: Progressive disability in multiple sclerosis (MS) occurs because central nervous system axons degenerate as a late consequence of demyelination. In animals, retinoid X receptor (RXR-γ) agonists promote remyelination. We assessed the safety and efficacy of a licensed non-selective RXR agonist as a remyelinating MS treatment.

Methods: In this completed double-blind phase 2a trial (CCMR One, ISRCTN14265371) people with relapsing remitting MS from two UK centres, aged 18-50 years, who had been on dimethyl fumarate for ≥6 months, were randomly assigned (1:1) bexarotene 300 mg/m^2 or placebo for 6 months, by independent staff. All trial participants and personnel were masked to treatment assignment. The primary endpoint was safety; 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, with prespecified MRI and visual evoked potential exploratory outcomes.

Findings: Between Jan 17th, 2017, and May 17th, 2019, 52 participants were randomised. All those on bexarotene experienced adverse events: central hypothyroidism (n=26, 100% v none on placebo), hypertriglyceridaemia (n=24, 92% v none on placebo), rash (n=13, 50% v 1, 4% on placebo) and neutropenia (n=10, 38% v none on placebo). Five participants on bexarotene and two on placebo discontinued study drug due to adverse effects. One episode of cholecystitis in a placebo-treated participant was the only serious adverse event. The primary efficacy outcome was not met. The unadjusted change in MTR was 0·25 (0·98) pu for submedian lesions in bexarotene-treated participants versus 0·09 (0·84) pu for those on placebo. The bexarotene-placebo difference in adjusted mean submedian lesional MTR change was 0·16 (0·25 vs 0·09 [95% CI -0·39, 0·71]) pu, p=0·554.

Interpretation: We do not recommend bexarotene as a treatment of multiple sclerosis because of its poor tolerability and negative primary efficacy outcome. However, statistically significant effects were seen in some exploratory imaging and electrophysiological analyses, suggesting that other RXR agonists might have a small biological effect that could be investigated in further studies.

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Research in context

Evidence before this study

We searched PubMed for articles published in English, between Jan 01, 2000, and Mar 01, 2021, reporting on phase 1, 2 or 3 MS remyelination clinical trials, using the terms "multiple sclerosis" OR "MS" AND "remyelination". We also searched the clinical trials databases clinicaltrials.gov and ISRCTN using the search term "remyelination". A number of clinical trials using a remyelinating drug to treat chronic and acute demyelinating injuries have been reported, but only one was published prior to commencement of CCMR One: the phase 2 study of GSK239512, a H_3 receptor antagonist, had shown a borderline significant improvement in the magnetisation transfer ratio (MTR) characteristics of acute lesions. Evidence emerging since then has included the phase 2 ReBUILD study of clemastine, which demonstrated a statistically significant improvement in the latency of the full-field visual evoked potential in people with relapsing MS and chronic stable optic neuropathy. Additionally, a phase 2 clinical trial (RENEW) of opicinumab (anti-Lingo1), showed an improvement in visual evoked potential latency using a per protocol analysis of participants with acute optic neuritis; though it did not reach its primary endpoint when deployed in a further phase 2 study (SYNERGY) using a multicomponent measure of disability.

Serial MTR has provided semi-quantitative *in vivo* measures of myelin content within white matter, grey matter, chronic and acute lesions. Meanwhile, analyses of visual evoked potentials have either centred on serial changes in those with stable, but prolonged, P100 latencies, or on those recovering from a recent bout of optic neuritis (in which case latencies for the unaffected contralateral eye have been used as a control). There is no consensus on the optimum endpoint to deploy in phase 2 remyelination trials.

Added value of this study

CCMR One is the first clinical trial to test the remyelinating potential of RXR-γ agonism, established in the laboratory, by investigating the safety and efficacy of bexarotene (an RXR agonist with activity against the α, β, and γ isoforms) in people with relapsing remitting MS. It is also the first clinical trial that has shown a remyelinating effect of a drug with converging evidence from both MRI and electrophysiological assessments. While this trial did not meet its primary efficacy endpoint – there was no statistically significant difference in adjusted

submedian lesional MTR change between bexarotene and placebo – in prespecified exploratory analyses it showed statistically significant treatment effects on lesional MTR in cortical grey matter, deep grey matter and the brainstem lesions. This trial also found electrophysiological evidence of remyelination in a prespecified exploratory analysis of bexarotene treated participants who had established demyelination in the visual pathway at baseline. Bexarotene was poorly tolerated, though some side effects (hypertriglyceridaemia and neutropenia) probably reflect agonism via other (RXR-α and β) pathways.

Implications of all the available evidence

We do not recommend bexarotene as a treatment of multiple sclerosis because of its poor tolerability and negative primary efficacy outcome. However, our results support the strategy of therapeutically enhancing remyelination by targeting the retinoid X receptor-γ pathway. They reinforce findings from the pathology literature that lesion remyelination is influenced by location and baseline tissue integrity, and this has important ramifications for other trials of putative remyelinating drugs. These data also support the use of visual pathway electrophysiological outcomes in future trials of remyelination. Further studies are needed to determine whether more selective RXR-γ agonists can replicate the beneficial effects without the tolerability and safety concerns that preclude the widespread use of bexarotene in MS.

Introduction

In multiple sclerosis (MS), which affects 2·8 million people worldwide and is among the commonest causes of disability in young adults, central nervous system inflammation leads to acute demyelination.[1] Although many licensed drugs reduce inflammation effectively,[2] they leave persistently demyelinated axons, which slowly degenerate through loss of trophic support, causing progressive worsening of disability.[3] An important unmet clinical need is a regenerative treatment to delay or prevent disability progression.[4]

The most effective strategy to preserve demyelinated axons is to enhance endogenous remyelination (reviewed[5]). This process – requiring the migration, proliferation and differentiation of oligodendrocyte progenitor cells (OPCs) – ultimately fails in most people with MS.[6,7] As OPCs are often found in chronically demyelinated MS lesions,[8] remyelination failure can be attributed in part to impaired OPC differentiation. Studies to identify therapies capable of enhancing this rate-limiting stage[9,10] have led to clinical trials.[11-13] Clemastine, for example, was first shown to stimulate *in vitro* OPC differentiation and ensheathment of conical micropillars,[10] and then improved the conduction of visual evoked potentials in people with MS and chronic stable optic neuropathy.[11]

Another positive regulator of OPC differentiation is the retinoid X receptor (RXR)γ,[14] which is expressed in remyelinated MS lesions in oligodendrocyte lineage cells. Inhibition of RXR-γ signalling inhibits differentiation of rodent and human OPCs; and the RXR agonist, 9-cis-retinoic acid, remyelinates both demyelinated cerebellar slice cultures, and focal toxin-induced demyelination in aged rats.[14] There are no licensed selective RXR-γ agonists;[15] however bexarotene, a non-selective agonist of the α, β, and γ isoforms, is licenced to treat cutaneous T-cell lymphoma.

There is no consensus on optimal endpoints or realistic treatment effects in trials of remyelinating drugs.[4] Magnetic resonance imaging sequences such as magnetisation transfer ratio (MTR) correlate with myelin content and to lesser degrees with axonal and glial density,[16,17] and allow feasible sample sizes in remyelination trials with estimated treatment effects.[18] Alternatively, the functional consequences of remyelination in the visual pathway can be assessed by visual evoked potentials.[5]

We undertook a phase 2 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 2 study conducted at the Cambridge University Hospitals NHS Foundation Trust and the University of Edinburgh Anne Rowling Regenerative Neurology Clinic. Initial eligibility criteria were that participants had relapsing remitting MS, were aged 30-50 years, had an Expanded Disability Status Scale (EDSS) score between 3·0 and 6·0, and had at least one relapse in the two years prior to screening, as well as ≥ 5 T2 hyperintense MS lesions on MRI. Four months into the trial, the eligibility criteria were changed following advice from the Trial Management Group, to drop the requirement for active relapsing disease and include younger and less disabled patients, to those aged 18-50 years, and with an EDSS from 0·0-6·0. Full selection criteria are given in the Appendix [page 1]. 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[19] – 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 (details available in the study protocol), additionally excluding those with significant cardiovascular disease or lymphopaenia ($<0{\cdot}7 \times 10^9$/L within 6 months of screening) in view of adverse events 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 National Research Ethics Service Committee (15/LO/0108). All participants gave written informed consent at enrolment.

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 visual evoked potentials were labelled with secondary codes that did not identify the trial participant, and were analysed at the end of the study. All data was stored in a commercial data entry system (Elsevier MACRO) hosted by the Cambridge Clinical Trials unit and cleaned, then locked before the treatment allocation code was broken by the trial statistician.

Procedures

The IMP was unmarked capsules of 75 mg bexarotene (Targretin®; Eisai Ltd) or placebo, 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. Participants were seen 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, patients were prescribed levothyroxine 50 to 100 mcg and the dose increased until FT4 normalised. Fenofibrate and thyroxine were stopped, per protocol, at month 6 with the IMP. If a participant developed neutropenia (<1·0x10^9/L), IMP doses were reduced to 200 mg/m^2 and, if persistent, to 100 mg/m^2.

MRI scans were performed at baseline and 6 months using one Siemens 3T Prismafit scanner (Siemens, Erlangen, Germany) per site with 20-channel head-neck coils at each site (see Appendix, Table 1, p.4). 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. 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 (see Appendices, p.3). 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 recordings were taken from each eye at each visit. VEP latency was defined by the P100 and values greater than 118 ms. 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.

Outcomes

The safety outcomes were adverse events and withdrawals attributable to bexarotene. 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. Prespecified exploratory electrophysiological outcomes 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, and those with a past history of optic neuritis, with a per-protocol analysis pre-specified if treatment non-adherence was greater than 10%. Other pre-specified endpoints were [1] the proportion of Gd-enhancing lesions present at month zero that progress to black T1 holes at month six; [2] the proportion of acute MRI lesions appearing on-trial that show an increase in MTR by month six; [3] the number of Gd-enhancing MRI lesions that appear on trial; [4] the change at month 6 in the MTR of all individual T2 and T1-hypointense lesions seen at baseline; [5] the change in MRI T1 brain volume; [6] the change in MTR of white and grey matter; [7] the change in MRI T2 lesion load; [8] peripheral immune cell populations before and after treatment; and [9] the change in EDSS over 6 months. A sub-group analysis of the primary efficacy outcome in those patients who developed grade 3 or 4 serum triglyceride increase was pre-specified.

Power calculation

We 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 and sensitivity analyses is described elsewhere.[18] 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 for a 1:1 allocation ratio sufficient to detect a 40% treatment effect, corresponding to a difference of 1·18 pu, with a standard deviation of 1.91 pu, giving a standardised effect size of 0.618. 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;[18] 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 models were estimated using restricted maximum likelihood (REML), but without the Kenward-Roger adjustment for degrees of freedom since applying the latter did not affect the results at a small enough decimal place to impact on reporting. For individually randomised observations we would not expect to have both non-significant treatment effects yet a significant difference between treatment effects (interaction), since in such contexts the standard error for the

interaction term will be higher than for the individual treatment effects. However, in this context, where patients and not lesions are randomised, the lesion-level treatment effects are necessarily between patient: active and placebo lesions can never occur within the same patient. However, sub- and supramedian lesions can both occur within the same patient, and since the interaction term is equivalently interpreted as the difference between sub- and supramedian lesions in active compared to placebo, it can be estimated with a strong within-patient component: this greatly reduces the interaction term standard error, permitting a smaller p-value than for the between-patient main treatment effects. 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. For EDSS, a corresponding multiple regression was checked using a non-parametric bias-corrected and accelerated bootstrap with 1000 replicates. For both regression and mixed models, residuals were examined for departures from Normality and homoscedasticity, and satisfied assumptions. Statistical methods to analyse the exploratory endpoints are described in the Statistical Analysis Plan. Analyses were carried out in Stata 16·1 (Stata Corporation, College Station, Texas, USA). Statistical significance refers to two-sided $p<0{\cdot}05$.

Role of the funding source

The funders of the study had no role in the study design, collection, analysis or interpretation of data, of writing the report, or in the decision to submit for publication. All authors had full access to all the data in the study. The corresponding author and AJC, had final responsibility for the decision to submit for publication.

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 1). 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 1) and their baseline characteristics are included in Table 1.

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).

All 26 (100%) bexarotene-treated participants developed central hypothyroidism (see p.7 of Appendix). 24 of these required thyroxine; 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 ($<1{\cdot}0x10^9$/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.

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 1) or new T2 hyperintense lesions at follow-up (7 lesions in 5 patients, see Appendix, Table 2, p.6) to allow analysis of the endpoints 1-3 listed above. We replaced endpoint 5, MRI T1 volume, with the more reliable Brain Parenchymal Fraction (see Table 3). The lesion masking prevented analysis of endpoint 5 [MRI T2 lesion load] and endpoint 8 will be reported in a later publication.

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 3, Figure 2A. 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 3, Figure 2B). 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 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 2C. 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). There was no significant treatment effect of bexarotene on all T2 lesions combined, brain parenchymal fraction or normal-appearing whole tissue MTR (Table 3).

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. In a 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 3, Figure 3. This difference remained statistically significant after excluding eyes affected by clinical optic neuritis within 5 years (adjusted latency difference was –4·75 (95% CI -8·80, -0·71) ms, p=0·032 in an ITT analysis, and -6·54 ms (95% CI, -10·62, -2·47), p=0·006 in the per protocol (PP) group). 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 (95% CI -5·75, 0·05) ms, p=0·054), but in the PP analysis a larger statistically significant adjusted difference (-4·02 (95% CI -7·27, -0·76) ms, p=0·015)) was seen; Figure 3.

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). Similarly, there was no treatment effect on change in logMAR 100%-contrast visual acuity between baseline and 6 months (adjusted bexarotene-placebo difference 0·03 (-0·03, 0·07), p=0·339).

Discussion

We do not recommend the use of bexarotene in people with MS. Bexarotene was poorly tolerated and the primary efficacy objective, using a 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.[14]

Bexarotene caused central hypothyroidism in all patients, raised triglycerides in 92%, headache in 54%, rash in 50% and neutropenia in 38%. The rates of hypothyroidism and raised triglycerides exceed those when bexarotene is used in cutaneous T cell lymphoma (30% and 74% respectively),[20] perhaps because of an interaction with dimethyl fumarate, whose effects on nrf2 transcription may additionally have been suppressed by bexarotene.[21] 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,[15] although thyroid dysfunction would remain a potential adverse effect of RXR-γ agonists.[22]

No previous trial has shown remyelination on both MRI and electrophysiological measures (reviewed by Lubetzki[4] and Cunniffe[5]). Mesenchymal stem cells led to improvements in VEP latency and visual acuity but not MTR.[23] Clemastine reduced VEP latency in eyes with chronic stable optic neuropathy but had no impact on MRI outcomes.[11] Anti-Lingo1 reduced VEP latency in acute optic neuritis in a per protocol analysis, but had no effect on MRI measures.[12] Small MTR increases were reported with an H3 receptor antagonist (in lesions).[13]

Importantly for the design of future trials examining remyelination in MS, this study demonstrates that MS lesions are heterogeneous in their capacity for remyelination in response to RXR agonists. There was greater remyelination in lesions that were more demyelinated at baseline. Also, grey matter plaques showed greater remyelination than those in white matter, which is consistent with the pathology literature.[24-26] 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. 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.[27] At 3T, FLAIR detects less than 7% of pure CGM lesions at post-mortem; it identifies no intracortical or purely subpial lesions.[28] The cortical GM lesion

results may therefore not be generalisable to all cortical lesions. We therefore recommend future phase 2 remyelination trials use both VEP and MRI outcome measures sensitive to grey matter lesions. 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. We believe the most useful patient population for phase 2 trials of remyelinating therapies of chronic lesions is inactive non-disabled relapsing-remitting multiple sclerosis, on immunotherapy, in whom there are most likely to be established demyelinating lesions with intact axons.

Limitations of our 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,[14] other mechanisms may be at play. Bexarotene may also have enhanced remyelination indirectly by increasing phagocytosis of myelin debris,[29] which inhibits OPC differentiation,[8] through the RXR-α pathway. We cannot exclude the possibility that thyroxine, used to treat 24 patients' hypothyroidism in the bexarotene arm, promoted remyelination,[30] although patients' T3 and T4 levels never rose above pre-treatment levels (see Appendix, p.7). Nevertheless, our data, together with other studies using therapies that target OPC differentiation,[11,12] 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.

Figure legends

Figure 1. Trial design. EDSS: expanded disability status scale.

Figure 2. 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. Dotted line represents the target difference in the power calculation. Pu: percentage unit; GM: grey matter; DGM: deep grey matter; WM: white matter. All are pre-specified endpoints: A is the eprimary efficacy endpoint, B-C are exploratory.

Figure 3. 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. Both are pre-specified exploratory endpoints.

Contributors

JWLB designed and wrote the trial protocol, recruited participants, was an evaluating physician, oversaw MRI data acquisition, analysed the data, and wrote the manuscript. NGC recruited participants, was an evaluating physician, oversaw VEP data acquisition, analysed the data, and wrote the manuscript. FP, BK, DM, RSS, JS, and CGWK analysed MRI data, and critically revised the manuscript. JJ, EN, and ZG were evaluating physicians, and critically revised the manuscript. DR, ORP, and JO were members of the trial steering committee, approved the protocol, oversaw trial safety, and critically revised the manuscript. CFFC and RJMF developed the preclinical scientific rationale for the study, advised on the protocol and critically revised the manuscript. CM handled the thyroid protocol and advised on cases. PDF handled the lipid protocol and advised on cases. AWM was responsible for VEP data acquisition and critically revised the manuscript. SC and PC were primary investigators, advised on the protocol, acted as evaluating physicians, acquired data, and critically revised the manuscript. DRA advised on the protocol, led the power calculations, wrote the statistical plan, did the primary analysis of the data, and critically revised the manuscript. DTC advised on the protocol, oversaw all aspects of the MRI data acquisition and analysis, and critically revised the manuscript. AJC designed and wrote the trial protocol, secured funding, evaluated and recruited participants, oversaw data collection and analysis, and wrote the manuscript. All authors had access to all the raw data, after the outcomes had been evaluated. AJC, NGC and JWLB verified the data.

Declaration of interests

JWLB reports personal fees from Biogen Idec for Real-World Evidence consultation, outside the submitted work. NGC reports grants from MS Society of GB, during the conduct of the study. FP has nothing to disclose. BK has nothing to disclose. JJ reports grants and personal fees from Sanofi, outside the submitted work. EN has nothing to disclose. ZG has nothing to disclose. DR reports grants and personal fees from Merck, grants and personal fees from Roche, grants and personal fees from Biogen, grants and personal fees from MedDay, grants and personal fees from Sanofi Genzyme, grants and personal fees from Novartis, personal fees from Janssen, personal fees from Celgene, grants from TG Therapeutics, and grants from Mitsubishi, outside the submitted work. ORP reports personal fees from Biogen, personal fees from Genzyme, personal fees from Merck, personal fees from Novartis, personal fees from

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Data sharing

We are committed to open access of trial data. The data for the primary efficacy endpoint is available from the EUDRACT website.

From our website: https://www-neurosciences.medschl.cam.ac.uk/jones-coles-group/ccmr-one-bexarotene-trial-datasets/ the following trial datasets (including data dictionaries) are available for researchers: deidentified participant data, primary efficacy endpoint dataset, VEP dataset and lesional MTR dataset. In addition, we will make these trial documents available: study protocol, statistical analysis plan, informed consent form. Access requests should be made to the CI (Alasdair Coles, ajc1020@medschl.cam.ac.uk). A signed data access agreement will be required and investigator support may be provided if part of an academic collaboration. All data will be available with publication, with no end date.

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