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[Intervention Protocol]

Cladribine for people with multiple sclerosis

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the short-term and long-term efficacy and safety of cladribine in people with any form of multiple sclerosis.

BACKGROUND

Description of the condition

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disorder of the central nervous system, characterised by inflammation, demyelination, and variable degrees of axonal loss and gliosis.

The aetiology of MS is not completely understood. We know that inflammation, which is considered a crucial component of MS etiopathogenesis, involves both T and B cells, with myelin sheath loss and the activation of inflammatory cascade and oligodendrocytes (Bhise 2016). Neurodegeneration is also an essential process in MS, closely linked to inflammation (Koudriavtseva 2016), which develops during the early stages of MS, and is related to disability accumulation and disease progression.

MS is the most frequently occurring demyelinating disease, particularly among young people (Miller 2012), with a prevalence that varies considerably, from high levels in North America and Europe (more than 100 per 100,000 population) to low rates in Eastern Asia and sub-Saharan Africa (2 per 100,000 population; GBD 2016). This leads to a significant economic burden on society and on the healthcare system.

MS is more prevalent in women than men, with a female:male ratio that varies by geographic region from 2:1 to 3:1, and has increased in the past decades (Golden 2017). Substantial epidemiological evidence supports the role of Epstein-Barr virus (EBV) infection (Laurence 2017), and smoking (Hedstrom 2019), as risk factors, while the role of vaccines, stress, and traumatic events has not been confirmed. Several epidemiological, immunological, and genetic studies have reported an association between vitamin D and MS, but the supplementation with vitamin D has not shown significant clinical benefit in people with MS (Jagannath 2018). It is also important that the likelihood of an individual developing MS is strongly influenced by ethnic background and family history, suggesting that genetic susceptibility is a key determinant of risk. The association between HLA-DRB1*15:01 and a high risk of MS has been known for decades and, more recently, over 100 loci have been firmly associated with susceptibility to developing MS (Hollenbach 2015).

MS-related neurological symptoms vary significantly among patients, and include visual disturbances, numbness/tingling sensations, weakness, bladder and bowel dysfunction, cognitive impairment, fatigue, and dizziness. Based on symptom onset and disease evolution, four MS phenotypes were initially described. The most frequent form (85%) is relapsing-remitting (RR-MS), where patients experience periods of new symptoms or relapses, which last at least 24 hours, followed by quiet periods of disease remission. More than 50% of people with RR-MS can develop a steady progression of symptoms over 15 years, with or without periods of remission, known as secondary-progressive MS (SP-MS; Lublin 1996; Weinshenker 1989). Some people with MS (15%) experience a gradual onset and steady progression of signs and symptoms, which is known as primary progressive (PP-MS). Finally, in some cases the MS clinical course can be relapsing-progressive (RP-MS). This classification has been recently reviewed: the RP-MS phenotype has been eliminated and the clinically isolated syndrome (CIS) has been added into the classification (Lublin 2014). CIS is a central nervous system demyelinating clinical

event, isolated in time, that is compatible with the possible future development of MS. There is a subgroup of people with RR-MS who have a more aggressive disease course characterised by a rapid accumulation of disability, despite treatment with one or more disease-modifying drugs. In the past, this disease phenotype was called 'aggressive' MS, and more recently has been called highly active MS (HAMS). It is generally agreed that the severe nature of this phenotype requires different treatment decisions. Unfortunately, there is no consensus on the definition of HAMS or on the treatment algorithm (Díaz 2019). MS prognosis is extremely variable and uncertain. In this regard, factors that may contribute to clinical evolution are age, sex, subtype of MS, magnetic resonance imaging (MRI) distribution of demyelinating lesions and spinal cord atrophy (Schlaeger 2014).

There is no definitive cure for MS. Steroid treatment during the relapse phase can accelerate clinical recovery, without a significant impact on long-term MS disability (Ciccone 2008). However, a wide range of immunomodulatory therapies or disease-modifying drugs have become available over the last few years. These drugs are promising, in light of the results of clinical trials, in reducing the possibility of relapses and the risk of disability progression. Currently disease-modifying drugs for MS approved by the European Medicine Agency (EMA 2017) include beta interferons, glatiramer acetate, dimethyl fumarate, fingolimod, teriflunomide, alemtuzumab, natalizumab, and more recently ocrelizumab and cladribine. The introduction of oral medications such as dimethyl fumarate, fingolimod, teriflunomide, and cladribine has been a crucial step in the history of this disease, allowing many patients an immediate improvement in terms of injection-related discomfort and day-to-day management and, possibly, reducing the costs of administering them.

A major objective at the time of the initial diagnosis is to arrest the disease at the early inflammatory stage, in the hope that this will also delay disease progression and minimise future disability. The new treatments have many indication for RR-MS, although there are relatively few options for progressive MS, that is, ocrelizumab for people with PP-MS. Other treatments for MS include symptomatic therapies focused on alleviating symptoms such as pain, fatigue, numbness, spasticity, and urinary disturbances.

Description of the intervention

Cladribine is a purine nucleoside analogue, which selectively depletes peripheral lymphocytes without a major impact on the innate immune system, in particular with a low level of influence on the activity of natural killer cells, eosinophils, macrophages and basophils. Chemically it mimics the nucleoside adenosine, but it is relatively resistant to de-amination by the enzyme adenosine deaminase. Cladribine undergoes intracellular phosphorylation by deoxycytidine kinase (DCK) and its active metabolite, cladribine triphosphate, accumulates within the cells and interferes with cellular metabolism, inducing DNA damage and apoptosis. Cladribine preferentially targets lymphocytes, producing rapid and sustained reductions in CD4+ and CD8+ cells, with rapid but more transient effects on CD19+ cells (Giovannoni 2017), relatively sparing other immune cells.

Cladribine was initially developed following research into the pathogenesis of the genetic disease adenosine deaminase deficiency (ADA), a severe immunodeficiency in children, resulting

from the accumulation of deoxyadenosine nucleotides in lymphocytes (Beutler 1992). Cladribine has been extensively used for decades, in a variety of haematological disorders, such as hairy cell leukaemia (Saven 1998), and B-cell chronic lymphocytic leukaemia (Juliussøn 1996). The drug has also been investigated for several other autoimmune disorders.

Considering the role of inflammation in MS, involving both B and T cells, and cladribine's action of depleting lymphocytes in the periphery and in the central nervous system, this treatment began to be evaluated (initially with parenteral administration), in people with relapsing and progressive forms of MS. Three randomised, double-blind, placebo-controlled, phase II-III trials have been conducted at the Scripps Research Institute (Beutler 1996; Rice 2000; Romine 1999), with promising results for reduction of relapses and disability progression. After these studies with parenteral cladribine administration, an oral tablet form was introduced. Cladribine has preferential and sustained activity in CD4+ and CD8+ T lymphocytes and B lymphocytes for more than 10 months following the last oral dose, probably affecting both resting and actively-dividing lymphocytes, including quiescent progenitor cells (Guarnaccia 2008). This allows the use of an annual short-course schedule. Its reduced effect on innate immune cells should preserve relative immunocompetence.

Over recent years many trials have been conducted with oral cladribine, involving people with different forms of MS. Trials such as ORACLE (investigating cladribine in people with CIS/early MS; Leist 2014), CLARITY (people with RR-MS; Giovannoni 2010; Giovannoni 2011), and CLARITY extension (a two-year extension of the CLARITY study; Giovannoni 2018), have shown encouraging results for relapse rates, disability progression, and MRI endpoints.

How the intervention might work

The cladribine molecule (also known as 2-CdA) is an adenosine analogue, acting as a prodrug. Its activity is dependent on the intracellular accumulation of its active triphosphate, which occurs preferentially in certain cell types. Three intracellular enzymes play a key role in determining the availability of its active form.

1. DCK (catalyses the first of three phosphorylation steps required to convert cladribine into its biologically-active nucleotide triphosphate, 2-CdATP);
2. 5'nucleotidase (5'NTase; catalyses the reconversion of phosphorylated cladribine to the cladribine nucleoside);
3. ADA (catabolic pathway through de-amination, even though cladribine is quite resistant to this enzymatic process).

The accumulation of the active 2-CdATP is dependent on the ratio of DCK, which creates it, to 5'NTase, which breaks it down (Kawasaki 1993).

The levels of DCK and the DCK:5'NTase ratio are high in T cells (CD4+, CD8+), B cells and dendritic cells. This results in selective accumulation of CdATP in lymphocytes, allowing cladribine to preferentially target these cells. As the active compounds increase within lymphocytes, there is inhibition of DNA synthesis and repair, disruption of cellular proliferation in actively-dividing lymphocytes, and apoptosis or autophagy, or both, of quiescent lymphocytes (Saven 1998). Alteration of cytokine patterns and induction of T-helper T-related cytokines may also contribute to the sustained effects of cladribine seen in clinical trials (Korsen 2015).

MS is an autoimmune inflammatory disorder of the central nervous system. Even if the pathogenesis is not completely clear, inflammation is considered to be an essential component of this disease, involving both T cells and B cells. Targeted reduction of cladribine-related lymphocyte subtypes is the basis of a possible new therapeutic approach.

Why it is important to do this review

Cladribine was approved in August 2017 in Europe for the treatment of highly-active RR-MS (EMA 2017), and in March 2019 in USA by the Food and Drug Administration (FDA; FDA 2019).

The EMA and the FDA withheld approval of this medication in 2010 to 2011 because of safety concerns (an increase in the frequency of cancer) in the CLARITY trial (NCT00213135; Giovannoni 2010). The ORACLE trial (Leist 2014), was terminated early by the study sponsor because of the negative regulatory decision on the CLARITY data. Results from the most recent trials resolved cladribine's initial problems, reassuring on safety and tolerance or tolerability profile. Data from the oral cladribine extension trial (Giovannoni 2018), and from the safety register, from the PREMIERE trial (NCT01013350), and re-analysis of the pivotal phase III CLARITY trial (Cook 2011; Pakpoor 2015), have indicated that oral cladribine is unlikely to be associated with an increased short- or medium-term risk of secondary malignancy. There may be a risk of Herpes Zoster infection, which is more frequently observed in those treated with cladribine tablets than with placebo, particularly in people with higher grades of lymphopenia (Cook 2009; Giovannoni 2010; Giovannoni 2013).

Even if this new treatment seems to be both effective and with a manageable side-effects profile, it is now important to evaluate the risk-benefit ratio of cladribine for people with MS, and to verify if this is confirmed in the long term. It is also essential to consider the risk-benefit ratio for cladribine in the different subtypes of MS. Only a few studies have investigated cladribine in CIS/early MS stages (the transition phase from CIS to MS in the ORACLE trial) and progressive MS (Beutler 1996; Rice 2000). Difficulties in distinguishing the precise clinical MS subtype in the early stage of this disease result in some cases in a delayed or inappropriate prescription of disease-modifying drugs. The possibility of finding a treatment that could be effective in different MS subtypes would be very valuable in the daily clinical approach to people with MS.

OBJECTIVES

To assess the short-term and long-term efficacy and safety of cladribine in people with any form of multiple sclerosis.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials (RCTs), their extension in open-label extension trial (OLEs), the first phase of cross-over trials and non-randomised prospective controlled studies. Inclusion will be regardless of their publication status and language of publication. We will exclude retrospective studies and studies without a comparison group.

Types of participants

We will consider participants with a confirmed diagnosis of MS or CIS, according to the published and internationally accepted criteria (McDonald 2001; Polman 2011; Thompson 2018). We will include people with any form of MS, regardless of age (inclusion of lower and higher age ranges, considering also people under the age of 18 and over the age of 65 years), sex, disability score, or duration of disease.

Types of interventions

Experimental intervention: oral or parenteral cladribine, alone or in combination, at any dose for any duration. We will accept concomitant interventions if they were used in all the comparison groups.

Comparator: no intervention, placebo or any other disease-modifying drugs.

Types of outcome measures

To address outcomes that are critical or important to decision making one of the review authors provided a predefined hierarchy list of common measures of MS disease to a panel consisting of the four review neurologists and four patients randomly selected, representing different levels of disease severity and gender. He asked each panel member to independently rate outcomes from the list, assigning a vote for each outcome on a scale of 1 to 9 (7 to 9: critical; 4 to 6: important; 1 to 3: of limited importance) according to the GRADE method (Schünemann 2013). After completion of the rating round, we computed the median of the votes and assigned a rate of importance to each outcome (see Appendix 1). We believe that this method takes into account the experience, the perspective and the values of the panel members and that this represents a transparent method for documenting and considering them. According to the GRADE method, we decided to include in the 'Summary of findings' tables a maximum of seven outcomes, that are all the outcomes rated as critical ($n = 6$) and the outcome rated as important with the highest vote. The remaining outcomes, including brain atrophy, which the review authors judged directly to be of limited importance without rating, will be discussed separately. In the final review we may reassess the relative importance of the outcomes in light of the available evidence.

Critical outcomes

1. The total number of serious adverse events. If not enough studies report the total number of serious adverse events and person-years, we plan to use the number of participants with at least one serious adverse event at the end of follow-up, as defined in the included studies.
2. The number of participants free from disability-worsening at 24 months after randomisation and at the end of follow-up. 'Disability-worsening' is defined as an increase in the expanded disability status scale (EDSS) of at least 1.0 point from baseline, sustained on subsequent visits for at least 12 weeks if the baseline score was 5.5 or less, or an increase of at least 0.5 points sustained for at least 12 weeks if the baseline score was more than 5.5 (EMA 2015).
3. The number of participants with 'no evidence of disease activity' at 24 months after randomisation and at the end of follow up. No evidence of disease activity is defined as a composite that includes the following parameters: freedom of relapse, EDSS

worsening, lack of new/enlarging T2 lesions on MRI, and lack of gadolinium-enhancing lesions on MRI (Lublin 2012; Rotstein 2015; Stangel 2015). It represent a comprehensive assessment of disease activity in routine clinical practice as it is used mainly to evaluate the disease-modifying treatment and eventually the following switch. However, there is no strictly accepted definition so far (Weinstock-Guttman 2018), and we will consider no evidence of disease activity as defined in the included studies.

4. Treatment withdrawal: the number of participants who withdrew due to any adverse event, lack of efficacy, lack of compliance or a combination of these causes at the end of the follow-up, from the total number of participants randomly assigned to each treatment arm. This outcome is used as a measure of global effectiveness at the end of the study period.
5. Quality of life, using validated health-related scales (e.g. Medical Outcomes Study Short Form-36 (SF-36; Ware 1992)) or MS-specific scales (e.g. MS Impact Scale-29 (Hobart 2001), Leeds MS Quality of Life (Ford 2001), MS Quality of Life-54 (Vickrey 1995)) or any other scale used in the trials, measured at the end of follow-up.
6. The number of participants with evidence of cognitive impairment at 12 months and at the end of follow-up assessed according to the following neurocognitive batteries: Brief Repeatable Battery of Neuropsychological Tests (BRB-N; Boringa 2001), Minimal Assessment of Cognitive Function in MS (MACFIMS; Benedict 2002), Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS; Langdon 2012), or the composite Multiple Sclerosis Cognitive Battery (MSCOG; Erlanger 2014, Sumowski 2018). Considering depression, anxiety and fatigue effect on cognition (Hughes 2019), we will report eventual simultaneous reporting of these conditions. We will consider validated scales: Hospital Anxiety and Depression Scale (Honarmand 2009), and Fatigue Severity Scale (Krupp 1989).

Important outcomes

1. The number of participants with at least one adverse event (describing type and severity), from the total number of participants randomly assigned to each treatment arm, at the end of follow-up.
2. The number of participants who experienced at least one new relapse over 12 months after randomisation and at the end of follow-up. 'Relapse' is defined as the appearance of one or more new symptoms due to MS, or the deterioration of pre-existing symptoms, persisting more than 24 hours in the absence of fever and preceded by a period of stability of at least one month (McDonald 2001).
3. The number of participants with new gadolinium-enhancing positive T1 weighted lesions or new/enlarging T2 weighted MRI lesions.

Outcomes of limited importance

1. The number of participants with evidence of brain atrophy as defined in the included studies, at 24 months and at the end of follow-up, compared with baseline.

Search methods for identification of studies

Electronic searches

The Information Specialist will search Cochrane Multiple Sclerosis and Rare Disease of the Central Nervous System's Trials Register, which, among other sources, contains trials from:

1. Cochrane Central Register of Controlled Trials (CENTRAL; most recent issue) in the Cochrane Library;
2. MEDLINE (Pubmed) (1966 to date);
3. Embase (Embase.com) (1974 to date);
4. CINAHL (EBSCOhost) (1981 to date);
5. Latin American and Caribbean Health Science Information Database (LILACS) (Bireme) (1982 to date);
6. World Health Organization (WHO) International Clinical Trial Registry Platform (ICTRP; apps.who.int/trialsearch/);
7. Clinical Trials registries (clinicaltrials.gov), for all prospectively registered trials.

Information on the Group's Trials Register and details of search strategies used to identify trials can be found in the Specialist Register Section within Cochrane Multiple Sclerosis and Rare Diseases of the Central Nervous System's website (msrdcns.cochrane.org).

The keywords that we will use to search for studies for this review are listed in [Appendix 2](#), [Appendix 3](#) and [Appendix 4](#).

Searching other resources

We will also use the following methods:

1. check reference lists of published reviews and retrieved articles for additional references;
2. contact trial authors and experts if the reported information is incomplete or for unpublished data;
3. screen the bibliographic references of selected trials to identify additional information;
4. search conference reports on neurology, MS societies (www.nationalmssociety.org), and congresses organised by the European Committee for Treatment and Research In Multiple Sclerosis (www.ectrims-congress.eu), and by the Americas Committee for Treatment and Research in Multiple Sclerosis (actrims.org);
5. contact biotechnology companies in an effort to identify further published and unpublished studies.

Data collection and analysis

Selection of studies

Three review authors (MM, MO and MVE) will independently select from the literature search titles and abstracts considered relevant for the review, obtaining the full text for further assessment to check whether the study meets our inclusion criteria. We will list those papers that do not meet the inclusion criteria for type of study, participants, or interventions, with the reason for exclusion in the 'Characteristics of excluded studies' table. We will resolve any disagreement about inclusion or exclusion by discussion among all the review authors.

Data extraction and management

Three review authors (MM, MGC and MVE) will independently extract data from the selected studies, using standardised forms, and will enter the data into [RevMan Web 2019](#) (RevMan Web).

We will extract the following information from individual studies:

1. publication details: (date, country, journal, authors);
2. study design and setting, inclusion/exclusion criteria, number of participants, characteristics of participants;
3. details of intervention (doses, frequency, scheme, length);
4. critical, important and of limited importance outcomes;
5. length of follow-up and trial period;
6. declarations of interest and funding source.

In open-label extension trials we will extract the number of participating centres and the number of participants who entered and completed the trial during different follow-up times. For each non-randomised study, we will record the analysis method that the study authors used to reduce confounding.

If there are disagreements, we will resolve them by discussion among all review authors.

Assessment of risk of bias in included studies

Three review authors (MGC, TAC, MO) will independently assess the risks of bias and the methodological quality of each included study, resolving any disagreement by discussion among all review authors. As we plan to include studies with different designs, we will use different 'Risk of bias' assessment tools. We will assess all the outcomes included in the 'Summary of findings' table for risk of bias both for randomised and non-randomised trials.

Randomised trials

For assessing risks of bias in randomised trials we will use the Cochrane 'Risk of bias 2' (Rob2) tool ([Sterne 2019](#)). This tool includes five bias domains:

1. bias arising from the randomisation process;
2. bias due to deviations from the intended interventions (effects of assignment and adherence to intervention);
3. bias due to missing outcome data;
4. bias in measurement of the outcome;
5. bias in the selection of the reported result.

Each bias domain includes signalling questions, having the following response options: yes, probably yes, probably no, no, and no information. Each trial will receive a 'Risk of bias' judgement for each outcome, that is, 'low risk of bias', 'some concerns' or 'high risk of bias' for each domain. We will calculate an overall 'Risk of bias' judgement from domain-level judgements. The overall 'Risk of bias' judgement for each study will be:

1. low risk of bias, if we rate the study at low risk of bias for all domains for the considered outcome;
2. some concerns, if we judge the study to raise some concerns in at least one domain for the considered outcome, but not to be at high risk of bias for any domain;
3. high risk of bias, if we judge the study to be at high risk of bias in at least one domain for the considered outcome, or if we judge

the study to have some concerns for multiple domains in a way that substantially lowers our confidence in the result.

To implement the Rob2 tool we will use [RevMan Web 2019](#).

We are going to quantify the effect of assignment to the interventions at baseline, regardless of whether the interventions are received as intended (the 'intention-to-treat effect'), while we will consider the effect of adhering to the intervention as specified in the trial protocol (the 'per-protocol effect') for serious adverse events and adverse events.

Cross-over trials

For assessing risks of bias in cross-over trials we will follow the Rob 2 ([Sterne 2019](#)), considering that we are going to include only the first phase.

Non-randomised studies

For assessing risks of bias in non-randomised studies of intervention we will use the Robins-I tool ([Sterne 2016](#)). This tool comprises the following seven bias domains: confounding, selection of participants into the study, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of reported result. We will judge the risk of bias for each domain in the tool as low risk of bias, moderate risk of bias, serious risk of bias, critical risk of bias, or no information. The response options for the list of the signalling questions for each domain are: 'yes', 'probably yes', 'no', 'probably no', and 'no information'. We will then assign an overall 'Risk of bias' judgement based on all seven domains, for each considered outcome.

1. Low risk of bias (the study is comparable to a well-performed randomised trial): we rate the study at low risk of bias for all domains.
2. Moderate risk of bias (the study appears to provide sound evidence for a non-randomised study, but cannot be considered comparable to a well-performed randomised trial): we rate the study at low or moderate risk of bias for all domains.
3. Serious risk of bias (the study has some important problems): we rate the study at serious risk of bias in at least one domain, but not at critical risk of bias in any domain.
4. Critical risk of bias (the study is too problematic to provide any useful evidence and should not be included in any synthesis): we rate the study at critical risk of bias in at least one domain.
5. No information on which to base a judgement about risk of bias: there is no clear indication that the study is at serious or critical risk of bias, and there is a lack of information in one or more key domains of bias.

We will specify in advance the following main potential confounding factors that could influence the intervention: age, sex, MS type and disease duration. Conversely, we will not identify at this stage possible co-interventions that could influence the effect of the intervention under study.

Measures of treatment effect

We will analyse data using [RevMan Web 2019](#). We will express the effect measure for dichotomous outcomes as a risk ratio (RR) with a 95% confidence interval (CI). For continuous outcomes, we will calculate mean difference (MD) or standardized mean

difference (SMD) with a 95% CI for the same continuous outcome measured with different scales. We will interpret SMD with Cohen's d according to usual cut-offs (0.2 small, 0.5 medium, 0.8 large), and if some scales increase or decrease with disease severity, we will multiply the mean values from one set of studies by -1 ([Higgins 2019](#)).

We will analyse the number of serious adverse events as count data in the form in which they are reported in each included study, and we will analyse them accordingly. We will decide whether to use rate data when the events being counted are rare (e.g. neoplasms).

Unit of analysis issues

We will include studies with a parallel-group design, with participants randomised to either intervention or comparison, with subsequent analysis at individual allocation level. We are going to consider data only from the first phase of cross-over trials as we are not able to measure the potential bias arising from subsequent phases. In fact MS may evolve over time or cladribine may have a carry-over effect not well known.

We will treat multi-arm trials as multiple, independent, two-arm trials in pair-wise meta-analysis. We will convert multi-arm trials involving cladribine at different doses compared to a control treatment into a single arm by merging doses and summing the number of events and the sample size to avoid double-counting of participants and potential correlation of results.

Dealing with missing data

We will contact the principal investigator of the primary study to request missing data or to query inconsistencies in the published articles. We will address missing data due to losses to follow-up or exclusions from the primary analysis with sensitivity analyses, imputing missing outcome data for participants within studies (such as worst-case or best-case scenarios), or imputing missing standard deviations, as described in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Deeks 2019](#)).

Assessment of heterogeneity

We will evaluate clinical and methodological heterogeneity in included studies by comparing the characteristics of participants (type of MS, age, gender, disease duration) and interventions (administration method, dosage and duration, control intervention, co-interventions). We will evaluate statistical heterogeneity among included studies using the Chi² test (significance level of 0.1) and the I² statistic ([Higgins 2003](#)). If the I² statistic is greater than 50%, indicating substantial heterogeneity, we will check the sources of potential clinical and methodological heterogeneity among the included studies ([Deeks 2019](#)).

Assessment of reporting biases

If the meta-analyses include more than 10 studies for an outcome, we will assess reporting biases according to the recommendations on testing for funnel plot asymmetry, as described in the *Cochrane Handbook for Systematic Reviews of Interventions*, Chapter 10 ([Sterne 2017](#)).

Data synthesis

We will pool results from clinically similar studies for meta-analysis if the methods and available data in each study are

sufficiently similar to enable pooling. We will perform separate meta-analyses for randomised and non-randomised trials, using a random-effects model in both analyses, because we assume that the studies will be both of different methodological quality and of different clinical severity syndromes, where a different efficacy of intervention could be expected. We will use adjusted estimates for non-randomised studies if available and we will keep them separate from estimates deriving from randomised trials. We will report the variables that have been used for adjustment in the included studies. We will conduct analyses using the inverse-variance random-effects method following [RevMan Web 2019](#). We will conduct meta-regression to examine the impact of covariates on the effect estimate (Deeks 2017) using [STATA 13](#) software.

We will consider as covariate the same subgroup planned in the subgroup analysis:

1. naïve versus other type of treatment used before cladribine treatment;
2. participants with RR-MS versus CIS versus PP-MS versus SP-MS;
3. participants under 18 years of age versus those aged 18 years and older;
4. different dosages of cladribine;
5. different routes of administration.

We will perform separate analyses for participants who had relapses or disability worsening measured at one year for the first outcome measure, at two years for the second, and at the end of the study for both.

Subgroup analysis and investigation of heterogeneity

We will conduct the following subgroup analyses if we identify a sufficient number of studies. We will consider the relevance of subgroups where at least 10 studies for a subgroup analysis are available.

1. Naïve versus other type of treatment used before cladribine treatment

2. Participants with RR-MS versus CIS versus PP-MS versus SP-MS
3. Participants under 18 years of age versus those aged 18 years and older
4. Different dosages of cladribine
5. Different routes of administration

Sensitivity analysis

We will re-analyse the data excluding studies judged to be at high risk of bias through both RoB 2 and Robins-I tools.

Summary of findings and assessment of the certainty of the evidence

We will assess the overall certainty of the evidence for critical and important outcomes according to the GRADE system. This takes into account issues not only related to internal validity (risk of bias, inconsistency, imprecision, publication bias) but also to external validity, such as directness of results. We will use methods and recommendations described in Chapter 14 of the *Cochrane Handbook for Systematic Reviews of Interventions* ([Schünemann 2019](#)). The GRADE system provides criteria for assigning grades of evidence for each outcome as high, moderate, low, or very low. The 'Summary of findings' table will present the main findings of the review in a transparent and simple tabular format, and we will report separate 'Summary of findings' tables for randomised and non-randomised studies. In particular, the tables will provide key information about the certainty of evidence, the magnitude of the absolute effect of the interventions examined, and the sum of available data on the critical outcomes and the first important outcome (seven in total).

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APPENDICES

Appendix 1. Hierarchy of outcomes according to their importance to assess the effect of cladribine in MS persons

Outcomes	MD_1	MD_2	MD_3	MD_4	MSP_1	MSP_2	MSP_3	Me- dian	Notes: 1-3 --> of limited impor- tance 4-6 --> important 7-9 --> critical	
Total number of SAEs	9	8	9	8	9	9	9	9	9.0	Critical
N° of participants free from EDSS worsening at 24 months	6	7	9	9	7	9	8	9	8.5	Critical
N° of participants with NEDA at 24 months	6	5	6	4	9	9	9	9	7.5	Critical
N° of participants who withdrew at the end of FU	7	7	9	8	8	5	1	9	7.5	Critical
Quality of life	9	9	6	7	9	7	7	7	7.0	Critical
N° of participants with cognitive impairment at 12 months	7	6	3	5	9	7	9	9	7.0	Critical
N° of participants with at least 1 AE at the end of FU	7	6	8	6	7	5	7	6	6.5	Important
N° of participants with at least 1 relapse over 12 months	7	6	6	5	4	8	8	6	6.0	Important
N° of participants with new gad T1 lesions or new/enlarging T2 MRI lesions	3	5	5	4	2	6	2	6	4.5	Important
N° of participants with brain atrophy at 24 months	Not rat- ed	Not rat- ed	Not rat- ed	Not rat- ed	Not rat- ed	Not rat- ed	Not rat- ed	Not rat- ed	Not rated	Of limited importance
MD: medical doctor; MSP: multiple sclerosis person; AEs: adverse events; EDSS: expanded disability status scale; FU: follow-up; MRI: magnetic resonance imaging; NEDA: no evidence of disease activity; SAEs: serious adverse events										

Appendix 2. CENTRAL search strategy

#1 MeSH descriptor: [Cladribine] this term only

#2 (cladribine):ti,ab,kw

#3 (leustatin):ti,ab,kw

#4 #1 or #2 or #3

#5 MeSH descriptor: [Multiple Sclerosis] explode all trees

#6 #4 and #5

Appendix 3. MEDLINE (PubMed) search strategy

((cladribine OR leustatin)) AND (((("Multiple Sclerosis"[mh]) OR ("Myelitis, Transverse"[mh:noexp]) OR ("Demyelinating Diseases"[mh:noexp]) OR ("Encephalomyelitis, Acute Disseminated"[mh:noexp]) OR ("Optic Neuritis"[mh])) OR (((("multiple sclerosis") OR ("neuromyelitis optica") OR ("transverse myelitis") OR (encephalomyelitis) OR (devic) OR ("optic neuritis")) OR ("demyelinating disease*") OR ("acute disseminated encephalomyelitis")))) NOT ((animals[mh]) NOT ((animals[mh]) AND (human[mh])))))

Appendix 4. Embase search strategy

((('encephalomyelitis'/exp) OR ('demyelinating disease'/exp) OR ('multiple sclerosis'/exp) OR ('myeloptic neuropathy'/exp) OR ('multiple sclerosis':ti,ab) OR ('neuromyelitis optica':ab,ti) OR (encephalomyelitis:ab,ti) OR (devic:ti,ab)) AND (((('cladribine'/exp) OR (leustatin:ab,ti) OR cladribine:ab,ti))) AND [humans]/lim AND [embase]/lim

CONTRIBUTIONS OF AUTHORS

MGC, MM, MO, MVE, and TAC drafted the protocol. All review authors participated in reviewing and editing the protocol and approved the protocol prior to publication.

DECLARATIONS OF INTEREST

MGC: none known
MM: none known
MO: none known
MVE: none known
TAC: none known

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