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Azathioprine for people with multiple sclerosis (Review)

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Ridley B, Nonino F, Baldin E, Casetta I, Iuliano G, Filippini G.
Azathioprine for people with multiple sclerosis.
Cochrane Database of Systematic Reviews 2024, Issue 12. Art. No.: CD015005.
DOI: 10.1002/14651858.CD015005.pub2.

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[Intervention Review]

Azathioprine for people with multiple sclerosis

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Editorial group: Cochrane Central Editorial Service.
Publication status and date: New, published in Issue 12, 2024.

Citation: Ridley B, Nonino F, Baldin E, Casetta I, Iuliano G, Filippini G. Azathioprine for people with multiple sclerosis. *Cochrane Database of Systematic Reviews* 2024, Issue 12. Art. No.: CD015005. DOI: 10.1002/14651858.CD015005.pub2.

ABSTRACT

Background

Multiple sclerosis (MS) is an immune-mediated, chronic, inflammatory demyelinating disease of the central nervous system, impacting around 2.8 million people worldwide. Characterised by recurrent relapses or progression, or both, it represents a substantial global health burden, affecting people, predominantly women, at a young age (the mean age of diagnosis is 32 years).

Azathioprine is used to treat chronic inflammatory and autoimmune diseases, and it is used in clinical practice as an off-label intervention for MS, especially where access to on-label disease-modifying treatments (DMTs) for MS is limited. Given this, a review of azathioprine's benefits and harms would be timely and valuable to inform shared healthcare decisions.

Objectives

To evaluate the benefits and harms of azathioprine (AZA) for relapsing and progressive multiple sclerosis (MS), compared to other disease-modifying treatments (DMTs), placebo or no treatment. Specifically, we will assess the following comparisons.

AZA compared with other DMTs or placebo as first-choice treatment for relapsing forms of multiple sclerosis
AZA compared with other DMTs or placebo for relapsing forms of MS when switching from another DMT
AZA compared with other DMTs or placebo as first-choice treatment for progressive forms of MS
AZA compared with other DMTs or placebo for progressive forms of MS when switching from another DMT

Search methods

We conducted an extensive search for relevant literature using standard Cochrane search methods. The most recent search date was 9 August 2023.

Selection criteria

We included randomised controlled trials (RCTs) lasting 12 months or more that compared azathioprine versus DMTs, placebo or no intervention in adults with MS.

We considered evidence from non-randomised studies of interventions (NRSIs) as these studies may provide additional evidence not available from RCTS.

We excluded cluster-randomised trials, cross-over trials, interrupted time series, case reports and studies of within-group design with no control group.

Data collection and analysis

We followed standard Cochrane methodology.

There were three outcomes we considered to be critical: disability, relapse and serious adverse events (SAEs, as defined in the studies). We were also interested in other important outcomes: quality-of-life (QoL) impairment (mental score), short-term adverse events (gastrointestinal disorders), long-term adverse events (neoplasms) and mortality.

Main results

We included 14 studies: eight RCTs (1076 participants included in meta-analyses) and six NRSIs (1029 participants). These studies involved people with relapsing and progressive MS. Most studies included more women (57 to 83%) than men, with participants' average age at the onset of MS being between 29.4 and 33.4 years.

Five RCTs and all six NRSIs were conducted in Europe (1793 participants); two RCTs were conducted in the USA (126 participants) and one in Iran (94 participants). The RCTs lasted two to three years, while NRSIs looked back up to 10 years. Four studies received some funding or support from commercial interests and five were funded by government or philanthropy; the other five provided no information about funding. There are three ongoing studies.

Comparison groups included other DMTs (interferon beta and cyclosporine A), placebo or no treatment. Below, we report on azathioprine as a 'first choice' treatment compared to interferon beta for people with relapsing MS. None of the studies reported on any critical or important outcome for this comparison for progressive MS. No study was retrieved comparing azathioprine to placebo or other DMTs for either relapsing or progressive MS. Furthermore, the NRSIs did not provide information not already covered in the RCTs.

Azathioprine as a first-choice treatment compared to other DMTs (specifically, interferon beta) for relapsing MS

- The evidence is very uncertain about the effect of azathioprine on the number of people with disability progression over two years compared to interferon beta (risk ratio (RR) 0.19, 95% confidence interval (CI) 0.02 to 1.58; 1 RCT, 148 participants; very low certainty evidence).

- Azathioprine may decrease the number of people with relapses over a one- to two-year follow-up compared to interferon beta (RR 0.61, 95% CI 0.43 to 0.86; 2 RCTs, 242 participants; low-certainty evidence).

- Azathioprine may result in a possible increase in the number of people with SAEs over two years in comparison with interferon beta (RR 6.64, 95% CI 0.35 to 126.27; 1 RCT, 148 participants; low-certainty evidence).

- The evidence is very uncertain about the effect of azathioprine on the number of people with the short-term adverse event of gastrointestinal disorders over two years compared to interferon beta (RR 5.30, 95% CI 0.15 to 185.57; 2 RCTs, 242 participants; very low certainty evidence).

We found no evidence comparing azathioprine to other DMTs for QoL impairment (mental score), long-term adverse events (neoplasms) or mortality.

Authors' conclusions

Azathioprine has been proposed as an alternative treatment for MS when access to approved, on-label DMTs is limited, especially in resource-limited settings. The limited evidence available suggests that azathioprine may result in a modest benefit in terms of relapse frequency, with a possible increase in SAEs, when compared to interferon beta-1b, for people with relapsing-remitting multiple sclerosis. The evidence for the effect on disability progression and short-term adverse events is very uncertain. Caution is required in interpreting the conclusions of this review since our certainty in the available evidence on the benefits and harms of azathioprine in multiple sclerosis is low to very low, implying that further evidence is likely to change our conclusions.

An important limitation we noted in the available evidence is the lack of long-term comparison with other treatments and the failure of most studies to measure outcomes that are important to people with multiple sclerosis, such as quality of life and cognitive decline. This is especially the case in the evidence relevant to people with progressive forms of multiple sclerosis.

PLAIN LANGUAGE SUMMARY

What are the benefits and risks of azathioprine (a type of medication that affects the body's immune response) for people with multiple sclerosis?

Key messages

- It is unclear if azathioprine (medication that affects the body's immune response) provides more benefits overall than other medicines for multiple sclerosis, like interferon (natural proteins made by the body to treat infection). Azathioprine may reduce the number of people experiencing relapses compared to interferon.

- People taking azathioprine over two years may be more likely to experience serious harmful effects than people taking interferon.

- Future studies should last longer than two years, focus on outcomes relevant for people with multiple sclerosis (quality of life, cognitive status) and include more people with progressive multiple sclerosis (whose symptoms gradually get worse).

What is multiple sclerosis?

Multiple sclerosis is a lifelong condition, affecting the brain and spinal cord. Its symptoms vary widely, can be mild or severe and include tiredness, pain, muscle cramps and reduction or loss of sensation and strength in parts of the body.

Multiple sclerosis typically affects young people, mainly women, and people are often first diagnosed when aged between 20 and 40 years. The most common form of MS is relapsing-remitting, where symptoms come ('relapse') and go ('remit'). 'Progressive' multiple sclerosis is when symptoms gradually get worse (i.e. there is no recovery or the body struggles to recover between relapses).

How is multiple sclerosis treated?

No treatment can cure multiple sclerosis, but many available medicines can reduce relapse frequency and slow disability progression. Azathioprine is a drug used in other diseases also caused by an impaired immune response. In countries where there are fewer treatments available, azathioprine is sometimes used to treat multiple sclerosis, even though it is not currently licensed for that purpose. A previous Cochrane Review found some evidence that azathioprine could be a possible treatment compared to interferon.

What did we want to find out?

We were interested in the benefits and harms of azathioprine, either as the first treatment choice ('first choice') or when other medicines did not work or were not wanted ('switching'), compared to other treatments.

We were also interested in the effects of azathioprine compared to placebo (dummy tablet) or no treatment.

What did we do?

We searched for studies comparing azathioprine with other medicines, placebo or no treatment. We compared and summarised the results of the studies and rated our confidence in the evidence, based on factors like study methods and size. We looked for randomised controlled trials (RCTs), where participants are assigned randomly to two or more groups. This way of conducting a study is the best way to reduce the impact of non-treatment factors that could influence the results. We also looked at non-randomised studies (NRS), meaning people were put in groups in a way that was not random or that the people chose which group they wanted to be in.

What did we find?

We found 14 studies (8 randomised trials and 6 NRS) that involved 2105 participants with relapsing-remitting and progressive multiple sclerosis. Ten studies included more women with multiple sclerosis (57 to 83%) than men. The average age of onset of MS was between 29.4 and 33.4 years. The non-randomised studies and five of the RCTs were conducted in Europe; two RCTs were conducted in the USA and one RCT was conducted in Iran.

The RCTs lasted up to three years, while the NRS looked back up to 10 years.

Four studies were funded by pharmaceutical companies, five by governments or charities, and five did not report this information.

Azathioprine as a 'first choice' treatment compared to other active treatments for relapsing multiple sclerosis

Compared to interferon, over two years of treatment:

- azathioprine may reduce the number of people with relapses;

- people taking azathioprine may experience more serious harmful effects;

- azathioprine may increase nausea or vomiting (or both); and

- the effect of azathioprine on worsening disability or short-term negative side effects is very uncertain.

We found no evidence comparing azathioprine to other treatments for the outcomes of quality of life, mental health, cancer or numbers of deaths.

We found no studies that looked at azathioprine as a 'first choice' treatment compared to other active treatments for progressive multiple sclerosis, or any studies that looked at azathioprine when 'switching' from other treatments, after they did not work or were not wanted, either for people with relapsing or progressive multiple sclerosis.

The non-randomised studies provided no additional information to that already provided by RCTs.

What are the limitations of the evidence?

We are not very confident in the evidence because:

- few studies, including relatively few people and very few events, are available;

- the quality of studies is not high; and

- the evidence does not cover all the comparisons we are interested in.

How up to date is the evidence?

Our searches were conducted up to 9 August 2023; the most recent included study is from 2014.

SUMMARY OF FINDINGS

Summary of findings 1. Azathioprine as a first-choice treatment versus other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis

Patient or population: adults (aged 18 + years) with relapsing multiple sclerosis

Settings: outpatient

Intervention: azathioprine as first-choice treatment

Comparison: interferon

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with other disease-modifying therapies (interferon)	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 2 years	69 per 1000	**56 fewer per 1000** (68 fewer to 40 more)	**RR 0.19** (0.02 to 1.58)	148 (1 RCT)	⊕⊖⊖⊖ Very low[a]	-
Relapse						
Assessed with: number of participants with clinical relapse **Follow-up:** 1 to 2 years	454 per 1000	**177 fewer per 1000** (259 fewer to 64 fewer)	**RR 0.61** (0.43 to 0.86)	242 (2 RCTs)	⊕⊕⊖⊖ Low[b]	-
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs **Follow-up:** 2 years	Not estimable (wide CIs) 0 events in the control group	Not estimable (wide CIs) 3 events in the intervention group	**RR 6.64** (0.35 to 126.27)	148 (1 RCT)	⊕⊕⊖⊖ Low[c]	-
Quality-of-life impairment (mental score)						

Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea or vomiting **Follow-up:** range from 1 to 2 years	25 per 1000	**108 more per 1000** (21 fewer to 4.653 more)	**RR 5.30** (0.15 to 185.57)	242 (2 RCTs)	⊕⊖⊖⊖ Very low[d]	-
Long-term adverse events (neoplasms)						
Assessed with: number of participants with neoplasms	-	-	-	-	-	Outcome not used in any included study
Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Outcome not used in any included study

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
Abbreviations
CI: confidence interval; **EDSS:** Expanded Disability Status Scale; **OIS:** optimal information size; **RCT:** randomised control trial; **RR:** risk ratio; **SAEs:** serious adverse events
[a]Downgraded one level for risk of bias: participants aware of treatment (open-label study); downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm.
[b]Downgraded two levels for imprecision: OIS not met. Not downgraded for risk of bias, although both studies were single-blind: when considering clinical relapse as an outcome, detection bias is unlikely.
[c]Downgraded two levels for imprecision: OIS not met, wide CIs including no difference, no events in control group; not downgraded for risk of bias: study is single-blind (participants aware of treatment), but detection bias for the outcome of SAEs is unlikely.
[d]Downgraded one level for risk of bias: both studies were single-blind (participants aware of treatment); downgraded one level for imprecision (OIS not met), and one level for inconsistency (considerable heterogeneity I^2 = 85%).

Summary of findings 2. Azathioprine when switching from a different disease-modifying therapy versus other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis

Patient or population: adults (aged 18 + years) with relapsing multiple sclerosis

Settings: outpatient

Intervention: azathioprine when switching from a different disease-modifying therapy

Comparison: other disease-modifying therapy

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with other disease-modifying therapies	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Relapse						
Assessed with: number of participants with clinical relapse	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Quality-of-life impairment (mental score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea or vomiting	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Long-term adverse events (neoplasms)						

Assessed with: number of participants with neoplasms	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study
Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Population (participants switching disease-modifying therapy) not included in any study

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
Abbreviations
CI: confidence interval; **EDSS:** Expanded Disability Status Scale; **SAEs:** serious adverse events

Summary of findings 3. Azathioprine as a first-choice treatment versus other disease-modifying therapies (interferon beta) for progressive multiple sclerosis

Patient or population: adults (aged 18 + years) with progressive multiple sclerosis

Settings: outpatient

Intervention: azathioprine

Comparison: other disease-modifying therapy

Outcome	Anticipated absolute effects *(95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with other disease-modifying therapies	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 2 years	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Relapse						

Assessed with: number of participants with clinical relapse	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Quality-of-life impairment (mental score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea or vomiting	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Long-term adverse events (neoplasms)						
Assessed with: number of participants with neoplasms	-	-	-	-	-	Intervention as first-choice treatment not included in any study
Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Intervention as first-choice treatment not included in any study

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
Abbreviations
CI: confidence interval; **EDSS:** Expanded Disability Status Scale; **SAEs:** serious adverse events

Summary of findings 4. Azathioprine when switching from a different disease-modifying therapy versus other disease-modifying therapies (interferon beta) for progressive multiple sclerosis

Patient or population: adults (aged 18 + years) with progressive multiple sclerosis

Settings: outpatient

Intervention: azathioprine when switching from a different disease-modifying therapy

Comparison: other disease-modifying therapy

Outcome	Anticipated absolute effects (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with other disease-modifying therapies	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study
Relapse						
Assessed with: number of participants with relapse	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study
Quality-of-life impairment (mental score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea or vomiting	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study
Long-term adverse events (neoplasms)						
Assessed with: number of participants with neoplasms	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study

Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Population (patients switching disease-modifying therapies) not included in any study

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
Abbreviations
CI: confidence interval; **EDSS:** Expanded Disability Status Scale; **SAEs:** serious adverse events

BACKGROUND

Description of the condition

Multiple sclerosis (MS) is an immune-mediated, chronic inflammatory demyelinating disease of the central nervous system (CNS). It typically affects young adults (predominantly women of childbearing age), with an average age at diagnosis of 32 years (Walton 2020). Its usual pathological features include multifocal areas of inflammation, demyelination, and axonal and neuronal loss, with astroglial scarring.

Although its course is variable, MS is commonly characterised by recurrent relapses or progression, or both. The condition ultimately leads to severe disability. Relapses are considered to be the clinical expression of focal inflammation and subsequent loss of the myelin sheath surrounding axons in the CNS. Relapses may be followed by complete or incomplete recovery.

In 85% of affected people, relapse is the only clinical expression during the early years of MS (the relapsing–remitting phase) (Lublin 2014). Subsequently, in an increasing proportion of patients, the disease course becomes progressive, with no recovery between relapses and constant worsening of disability; this is known as secondary progressive MS. In about 10% to 15% of people affected by MS, the progressive course is not preceded by relapses (Miller 2007); this is known as primary progressive MS. Approximately 40% of people with primary or secondary progressive MS show relapses during the course of the disease (Paz 2015). After the introduction of disease-modifying drugs, the risk of conversion to a progressive course has been shown to be reduced compared to untreated patients (Brown 2019; Confavreux 2000; Miller 2007).

The classification of MS into relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS) and progressive relapsing MS (PRMS) (Lublin 1996) has been used for over 20 years in clinical research and regulatory procedures regarding disease-modifying treatments (DMTs) for relapsing MS. This classification has recently been reviewed (Lublin 2014) and the concept of “disease activity” has been introduced, based on the presence of clinical relapse or new lesions identified by magnetic resonance imaging (MRI). Active forms of MS occur when the inflammatory process is ongoing, sometimes without corresponding clinical manifestations if the inflamed region of the CNS is clinically silent. The 2013 updated classification of MS includes: active or inactive relapsing MS (RMS), with or without worsening; and active or inactive primary or secondary progressive MS, with or without progression. Two new forms were also added: clinically isolated syndrome (CIS) and radiologically isolated syndrome (RIS), and the definition of PRMS was abandoned.

Although it is not a common condition, MS represents a substantial health burden globally as it affects young adults during their working lives (Walton 2020). The global incidence and prevalence of MS are increasing. From 1990 to 2016, the age-standardised prevalence of MS increased by 10.4% GBD 2019. About 2.8 million people worldwide are affected by MS (35.9 per 100,000 population) and this figure has increased by about 500,000 since 2013. The global pooled incidence rate is 2.1 per 100,000 persons/year (GBD 2019; Walton 2020).

Currently, no treatment is available to stop the natural course of MS towards progressive disability. Available MS treatments are based on immune-modulating or immune-suppressing drugs, also called DMTs, to distinguish them from symptomatic drugs for the treatment of specific symptoms of MS (e.g. urinary incontinence or retention, muscular spasms, painful sensitive symptoms). Relatively few studies directly compare different DMTs or assess the sequential use of specific DMT combinations. Therefore, clinical practice guidelines on MS treatment usually do not recommend one DMT over the other. The variability of recommendations amongst guidelines concerning specific drugs in part reflects differences in the decisions by regulatory drug agencies and in regional and local health policies (Ghezzi 2018). Recent approvals of ocrelizumab, siponimod, ozanimod and cladribine mean that, for the first time, people with progressive forms of MS have different treatment options.

A previous Cochrane review appraised the available evidence from randomised controlled trials (RCTs) on the efficacy and safety of azathioprine (AZA) compared to placebo in MS (Casetta 2007). The authors concluded that AZA is an appropriate maintenance treatment for frequently relapsing patients with MS and may be a fair alternative to interferon beta for treating MS, although a potentially increased risk of malignancy associated with high cumulative doses of AZA could not be excluded.

Description of the intervention

Azathioprine is a purine analogue affecting DNA replication through inhibition of the synthesis of nucleic acids. It is metabolised by the enzyme thiopurine methyltransferase (TPMT). Some people have very low or absent TPMT levels due to a homozygous mutation of the gene coding for the enzyme; these individuals should not be treated with AZA because the drug is not metabolised, which exposes them to the risk of severe bone marrow suppression. Genetic screening for TPMT deficiency is therefore warranted before starting treatment.

Azathioprine was produced in the mid-1950s (Elion 1993), and by 1960 it was used in clinical practice (Rundles 1961). Because of its favourable therapeutic index over other traditional immunosuppressants, AZA is frequently used as a corticosteroid-sparing agent and as monotherapy to treat several chronic inflammatory and autoimmune diseases (e.g. rheumatoid arthritis, inflammatory bowel disease, Crohn’s disease, systemic lupus erythematous, myasthenia gravis, malignancies and other autoimmune conditions) (McWilliam 2020).

Azathioprine is administered orally as 25 mg or 50 mg tablets. The starting dose in MS is 1 mg per kilogram of body weight per day (mg/kg/day), given as a single dose once or twice daily, gradually increased over four to six weeks to a maintenance dose of 2.5 mg/kg/day to 3 mg/kg/day (100 mg/day to 150 mg/day) and adjusted according to regular monitoring (every two to three months) of white blood cell count. In the case of a decrease in white blood cell count or lymphocyte count, a dose reduction of between 25 g and 50 mg is required. AZA is a slow-acting agent, with therapeutic response being observed after at least three months (and up to six months) of treatment. Side effects are reported in about 10% to 28% of patients treated with AZA, 50% to 80% of whom discontinue the treatment. The most common clinical side effects occurring during treatment, particularly at the beginning of treatment, are gastrointestinal (anorexia, nausea, vomiting) (Lee 2015). Gastrointestinal side effects are experienced by about 12%

of patients with MS treated with AZA (Invernizzi 2008); they can be prevented by taking the drug close to meals.

Dose-dependent, reversible leukopenia and thrombocytopenia may be a consequence of bone marrow suppression in 27% and 5% of people treated with AZA, respectively. Bacterial, viral and fungal infections associated with immunosuppression occur in about 9% of people treated with azathioprine (Huskisson 1984; Lallana 2011; Weinshilboum 1980). Long-term adverse events may include the risk of malignancy (lymphoma, skin cancer), although data on patients with MS are inconsistent (Amato 1993; Lhermitte 1984). Evidence from transplant recipients treated with AZA suggests that cancer risk may be dose-related, although such a possibility is still debated (Na 2016; Pasternak 2013).

How the intervention might work

The pathophysiology of MS supports the use of immunosuppressive medications (Compston 2002; Massacesi 2002). T-cell-mediated immune response has a central role in the pathogenesis of MS. Indeed, an increased number of T-lymphocytes, specific for myelin and other CNS antigens, has been observed in people with MS; these, together with B-cells, are thought to initiate and perpetuate the immune component of the disease, as suggested by the presence of oligoclonal bands of immunoglobulin G in the cerebrospinal fluid of people with MS.

In the pathophysiological process of tissue damage in MS, T-cell death through apoptosis (namely, activation-induced T-cell death) is involved. Evidence suggests that the elimination of autoreactive lymphocytes through apoptosis is reduced in people with MS, thereby maintaining a chronic cycle of inflammation (Ruggieri 2005). Therefore, drugs acting as modulators of apoptosis may be of therapeutic value (Zipp 2000).

In vitro studies show that AZA-induced apoptosis can be observed particularly on CD45RO, a specific subset of memory T-cells considered to be key effectors in autoimmune diseases such as inflammatory bowel disease (Tiede 2003; Zipp 2000). Such a mechanism, which is shared by other agents effective in MS (e.g. glatiramer acetate), could explain the immunosuppressive effects of AZA and its therapeutic action in MS through the elimination of pathogenic memory T-cells, and the subsequent reduction of tissue damage and therefore less severe disease (Ruggieri 2005; Zipp 2000). AZA shows an immunosuppressive activity due to the interference with nucleic acid synthesis during the cellular multiplication that follows B- and T-cell activation. Moreover, the purine antagonist effect inhibits the synthesis of RNA and DNA during replication of nucleic acid and the T-cell-dependent antibody-mediated response (Invernizzi 2008).

By suppressing the activation of the RAC1 gene, coding for the RAC1 protein, AZA and its metabolites determine the apoptosis of peripheral T-cells. This process also interferes with the activation of the CD28 receptor, a crucial component for initiating and regulating the immune response, in that it acts as a co-stimulator of alloreactive T-lymphocytes, mediated by the enzyme Rac1 GTP-ase (Tiede 2003). These observations are confirmed by studies on people with Crohn's disease and RAC1 has also recently been exploited as a therapeutic target for cancer treatments (Cannon 2020; Tiede 2003).

Why it is important to do this review

Azathioprine is not approved by the US Food and Drug Administration (FDA) for the treatment of MS, although in some European countries it is used and reimbursed (AIFA 2021; Hommes 2004; Kieseier 2010). In clinical practice, AZA has been used worldwide to treat MS for over 40 years. DMTs offer a broad spectrum of treatment options, although the most effective drugs are not always well tolerated and their cost may represent a substantial barrier to their use in settings with budget constraints (Zeineddine 2020). Recently published guidelines include off-label use of AZA for people who do not have access to approved DMTs amongst therapeutic options (Rae-Grant 2018; Yamout 2019). A global survey by the Multiple Sclerosis International Federation involving 89 countries showed that interferon beta is the most widely used on-label treatment, and azathioprine is used off-label to treat MS in 67% of the surveyed countries (Laurson-Doube 2021).

A previously published Cochrane review on the efficacy and safety of AZA versus placebo in people with MS included only RCTs, and concluded that AZA may be an alternative to interferon beta, with a favourable benefit-to-risk ratio (Casetta 2007). The authors suggested that a goal for future research would be direct comparison of AZA with interferon beta and that a potentially increased risk of malignancy associated with cumulative doses of AZA above 600 g could not be excluded.

Ensuring timely access to safe and effective treatments for people with MS is warranted. Since AZA has long been used off-label in people with MS, an updated systematic review of the available evidence on its efficacy and safety would be valuable to inform shared healthcare decisions by practitioners, policymakers, people with MS and their families.

As has been noted in regard to other off-label treatments used in MS, like rituximab (Greenflield 2018), given that patent protection of AZA has expired, it is unlikely that registered clinical trials to broaden the indication of AZA to MS will ever be undertaken, and it is therefore unlikely that new evidence on benefits and harms of AZA for MS will be provided by RCTs. As such, we decided to add non-randomised studies to the review (which was previously restricted to RCTs), in order to widen the evidence base, and to use the Risk Of Bias in Non-randomised Studies of Interventions tool (ROBINS-I) to critically evaluate the validity of non-randomised studies.

We considered that a new review was more appropriate, rather than updating the previous Cochrane review (Casetta 2007), because changes we wanted to make to the review methods were substantive. Several new DMTs have been approved since 2007 to treat relapsing and progressive MS. Therefore, we added new comparisons including all DMTs that were in use at mid-March 2022. We also included important new outcomes that were not addressed in the original review (i.e. serious adverse events, quality of life, long-term adverse events, common infections, cancer, MRI (magnetic resonance imaging) outcomes, mortality).

OBJECTIVES

To evaluate the benefits and harms of azathioprine (AZA) for relapsing and progressive multiple sclerosis (MS), compared to other disease-modifying treatments (DMTs), placebo or no treatment. Specifically, we will assess the following comparisons.

- AZA compared with other DMTs or placebo as first-choice treatment for relapsing forms of multiple sclerosis
- AZA compared with other DMTs or placebo for relapsing forms of MS when switching from another DMT
- AZA compared with other DMTs or placebo as first-choice treatment for progressive forms of MS
- AZA compared with other DMTs or placebo for progressive forms of MS when switching from another DMT

METHODS

Criteria for considering studies for this review

Types of studies

We included parallel randomised controlled trials (RCTs) in this review. We also included controlled non-randomised studies of interventions (NRSIs) since, given the status of AZA as an off-label treatment, it is possible they may represent the only or best available evidence if evidence from RCTs is minimal or lacking, for example, for long-term and rare adverse events, and especially (but not only) for the rare subtype of progressive MS. The controlled NRSI study designs that we included were studies with between-group designs, open-label extension studies, cohort studies and case-control studies.

We did not include cluster-randomised or cross-over trials to evaluate treatment with AZA in people with MS. We excluded case reports and studies of within-group design, for example, before-after (pre-post) studies with no control group, or interrupted time series.

Given the natural course of MS and the timescale of the expected effects of AZA on efficacy outcomes (e.g. disability progression, frequency of relapse), we considered only studies with a follow-up of 12 months or longer.

Types of participants

We included adult participants (aged 18 years or older) of either sex, who were treatment-naive (i.e. had received no treatment) or non-responsive to treatment with DMTs. We accepted the trials' definitions of 'non-responsive'. We included studies adopting any diagnostic criteria for MS. We included all types of MS (i.e. RRMS, SPMS and PPMS, regardless of disease duration and degree of disability).

Types of interventions

Experimental intervention

We considered treatment with AZA either as monotherapy or in combination with other treatments, regardless of dose, frequency of administration or disease duration. We considered AZA in combination with other treatments, where such treatments were used in all comparison groups. We included studies assessing AZA as a first-choice treatment in people with MS, as well as those investigating switching from a previous different DMT, regardless of the reason for switching, or method or timing of the switching. 'First choice' refers to chronological order of treatments used, i.e. the first treatment used by a previously untreated patient, and does imply not a ranking between treatments.

Comparison intervention

The comparator interventions were no intervention, placebo or any other DMT.

Types of outcome measures

We identified an initial list of the outcomes for this review, which we subsequently refined with the input of a multi-stakeholder guideline development group (including consumers, advisory groups, clinicians and other healthcare professionals with experience in the field of MS) in order to identify the most relevant (critical and important) outcomes for both patients and clinicians. We did not include studies if they did not report any of our outcomes of interest. We assessed adverse events using an exploratory approach (Peryer 2023), i.e. we included the short- and long-term adverse outcomes that were reported in the included studies.

Primary outcomes

We identified the following as critical outcomes.

- Disability

Number of people with MS with sustained disability worsening based on clinical follow-up visits at 24 months or more after randomisation. Worsening is defined as at least one increased point on the Expanded Disability Status Scale (EDSS) (Kurtzke 1983), or a 0.5-point increase if the baseline EDSS was greater than 5.5; this increase must have been confirmed in two consecutive clinical examinations separated by an interval of at least six months free from relapse and carried out by the same physician. EDSS is an ordinal scale where 0 is normal, 3 indicates mild disability, 6 indicates care requirement, 7 indicates wheelchair use and 10 indicates death from MS. An advantage of EDSS over other disability measures is its international acceptance (e.g. by the EMA (EMA 2015)) as a primary end point in clinical trials. It is also widely used in trials, enabling cross-study comparisons (Meyer-Moock 2014).

- Relapse

Number of participants with clinical relapse based on clinical follow-up visits at 12 months or more after randomisation. 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).

- Serious adverse events (SAEs)

Number of participants with SAEs, defined according to the authors of the included studies. Had an insufficient number of studies reported the total number of SAEs and person-years, we would have used the number of participants with at least one SAE as defined in the study. We specified individual SAEs where there was sufficient information available.

Secondary outcomes

We identified the following as important outcomes.

- Quality-of-life impairment (mental score)

Number of participants reporting quality-of-life impairment, assessed according to validated measures, amongst which

the Multiple Sclerosis Quality of Life-54 (MSQOL-54) is a multidimensional health-related quality-of-life measure (Vickrey 1995). The MSQOL-54 includes the generic 36-Item Short Form Survey instrument, supplemented with 18 MS-specific items, based on expert opinion and literature review. There is no single overall score for MSQOL-54. Two summary scores — physical health and mental health — can be derived from a weighted combination of scale scores (scale scores range from 0 to 100 and an increase in the score indicates improved quality of life).

Where the number of participants with quality of life impairment was not available, we considered the mean change in the subscores of the quality of life measure. We prioritised the mental QoL score as it explores cognitive, social and psychological/emotional functions that may be impacted by the diagnosis of MS itself and by physical disability.

- Short-term adverse events

Number of participants with drug-specific short-term adverse events. A short-term adverse event is defined as a problem caused by a treatment that usually goes away after treatment ends (NCI 2021). We identified gastrointestinal disorders as an important priority outcome.

- Long-term adverse events

Number of participants with drug-specific long-term adverse events, as reported in the included studies. Long-term adverse events are defined as problems caused by a treatment that may continue for months or years (NCI 2021). Such adverse events may be associated with dose accumulation of AZA. We identified neoplasms as an important priority outcome.

- Mortality: overall number of deaths

We identified the following as additional important outcomes.

- Other short-term adverse events: number of participants with drug-specific short-term adverse events, including immune system disorders, skin and subcutaneous tissue disorders and hypersensitivity reactions
- Other long-term adverse events: number of participants with drug-specific long-term adverse events, including infections and infestations (viral, bacterial, or fungal), blood and lymphatic system disorders, gastrointestinal disorders, hepatobiliary disorders, immune system disorders, skin and subcutaneous tissue disorders, CNS disorders
- Adverse events: number of participants with any adverse event, regardless of severity. We included clinical as well as instrumental adverse events, as defined in the studies.
- Quality-of-life impairment (physical score): number of participants reporting quality-of-life impairment, according to the MSQOL-54 physical domain
- Annualised relapse rate (ARR): mean number of new relapses per participant, adjusted for the duration of follow-up to annualise it
- Cognitive decline: number of participants with cognitive worsening assessed according to validated neurocognitive batteries for MS, for example, the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (Benedict 2020; Langdon 2012)
- New or enlarging T2-weighted MRI lesions: number of participants with new or enlarging T2-weighted MRI lesions at 12 months or longer after randomisation
- New gadolinium-enhancing positive T1-weighted MRI lesions: number of participants with new gadolinium-enhancing T1-weighted MRI lesions at 12 months or longer after randomisation
- Treatment discontinuation due to adverse events: number of participants who discontinued treatment due to adverse events, regardless of their severity

Search methods for identification of studies

All searches were designed and conducted by Chiara Bassi and Maria Domenica Camerlingo, Information Specialists for Cochrane Multiple Sclerosis and Rare Diseases of the CNS, with input from Robin Featherstone, Information Specialist, Cochrane Central Executive Team.

Electronic searches

We identified eligible study references through systematic searches of the following bibliographic databases (see Appendix 1) on 18 March 2022 and topped-up on 9 August 2023.

- Cochrane Central Register of Controlled Trials (CENTRAL, Issue 8, 2023) in the Cochrane Library (searched 18 March)
- MEDLINE PubMed (1966 to 9 August 2023)
- Embase (1974 to 2023 week 32)

We did not apply any search limitations with respect to study outcomes, methods of analysis or language. We included full-text publications, results published in non-commercial trial registries (e.g. ClinicalTrials.gov) and abstracts, whenever sufficient information was available on study design, characteristics of participants, interventions and outcomes.

Searching other resources

We searched for ongoing studies in the following additional sources (see Appendix 1) on 18 March 2022 and again on 9 August 2023:

- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP, apps.who.int/trialsearch); and
- US National Institutes of Health clinical trial register (ClinicalTrials.gov, www.clinicaltrials.gov).

We checked reference lists of all the included studies and any relevant systematic reviews identified for additional references to studies. We examined any relevant retraction statements and errata for the included studies. We searched for NRSIs according to the methods described in Section 24.3 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Reeves 2020).

Data collection and analysis

Selection of studies

Four review authors (FN, EB, BR, IC) independently screened the titles and abstracts of the search results and discarded studies that were clearly not relevant. The same authors then independently assessed all potentially relevant full text reports for eligibility. At both stages, any disagreements were resolved by discussion.

Three review authors (FN, EB, BR) compared multiple reports of the same study and used the most comprehensive report. They linked

multiple publications as companion reports but excluded true duplicates. Discrepancies were resolved by discussion. We report a selection of studies excluded at full-text stage in the Characteristics of excluded studies table, with our reasons for exclusion.

We considered abstracts and full texts in all languages for inclusion. All potentially eligible non-English-language abstracts were progressed to full-text review, with methods translated for eligibility consideration and the full text translated for data extraction. We report details of the included studies in the Characteristics of included studies table. We created a PRISMA flow chart to report the study selection process (Page 2021).

Data extraction and management

We conducted data management and extraction in accordance with the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020). Two pairs of review authors (from FN, BR, EB, IC and GI) independently extracted data from the studies included in the analysis using a predefined data extraction form in a Microsoft Excel spreadsheet, which we piloted with two RCTs and two NRSIs (Data Extraction Pilot Spreadsheet). We resolved disagreements by discussion. Where necessary, we consulted a third review author (GF). Data were managed and synthesised using RevMan Web (RevMan Web 2020). One review author (BR) transferred data to RevMan Web, while two review authors (IC, GI) double-checked the transferred data for accuracy by comparing the data presented in the systematic review with the data extraction form.

We extracted the following data from each included study.

- Study details: first author or acronym; year of publication; number of centres and location; study setting; study duration (total study duration, recruitment stage and follow-up); type of publication (full-text publication, abstract publication, unpublished data)
- Study design (RCT or NRSI); for NRSI, type of design; inclusion and exclusion criteria; number of participants in each arm; number of withdrawals; early termination of trial
- Participants: age; sex; diagnostic criteria; type and duration of MS; important baseline data (EDSS score; proportion of participants with previous use of DMTs; MRI brain lesions)
- Interventions: whether participants are treatment-naive or switching from a different treatment; comparison(s); concomitant medications
- Data analysis: type of estimate(s) provided; subgroup analysis, if performed
- Outcomes: primary and secondary outcomes specified and collected; method of outcome measurement; outcome time points reported; for NRSIs, confounding factors for which the study authors performed adjustment
- Disclosure of interests of study authors; funding source of the study

For continuous outcomes, we extracted the means and standard deviations of the comparison groups, where possible. We extracted arm-level data where possible. Where arm-level data were not available, we extracted effect sizes. We extracted data at the authors' defined time points.

We also retained studies where the population was mixed in terms of including people with both relapsing and progressive forms of multiple sclerosis, but data were not presented in subgroups. We applied a threshold of 50% to determine if the study should be considered as one subtype or the other.

Randomised controlled trials

Two review authors (EB, FN) independently assessed the risk of bias in each included study using the first version of the Cochrane risk of bias tool, RoB 1 (Higgins 2017). The recommended two-part tool to assess the risk of bias addresses specific domains of sequence generation and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), and selective outcome reporting (reporting bias), and problems not covered elsewhere (other bias). In the first part of the tool, the assessor describes what was reported to have occurred in the study, while in the second part, a judgement (low, high, or unclear) is provided about the risk of bias for each domain. To arrive at an overall risk of bias judgement for each RCT, we considered random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessor, incomplete outcome data and selective outcome reporting, and classified a study as at low risk of bias when we judged all the domains as at low risk of bias, high risk of bias when we judged at least one domain as at high risk of bias, and unclear risk of bias when we judged any of the domains as at unclear risk of bias, provided that all the remaining domains were at low risk of bias. We resolved any disagreements between the review authors by discussion.

Non-randomised studies of interventions

Two review authors (EB, IC) independently assessed the risk of bias using the ROBINS-I tool for NRSIs (version August 2016) (Sterne 2016). We defined our generic target trial as comparing AZA versus placebo or versus other DMTs for the treatment of people with MS. We therefore used the ROBINS-I analogue of starting experimental intervention versus starting control intervention to evaluate the risk of bias. The ROBINS-I tool includes the following 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 assigned an overall risk of bias judgement for each outcome based on the worst assessment across all bias domains, using the recommended levels (low, moderate, serious or critical risk of bias, or no information) (Sterne 2016). We resolved any disagreements between the review authors by discussion.

For each NRSI, we used the 'Risk-Of-Bias VISualization' (robvis) tool to create the risk of bias graphs (McGuinness 2021). We assessed whether the authors of the study considered the following potential confounders, if they were controlled for, and which method (statistical adjustment) was used by the authors to reduce confounding.

- Confounding by indication (pre-intervention confounder) when starting treatment in treatment-naive people with MS. In this scenario, more severe cases (e.g. severity determined by number of relapses in the previous year) are likely to be assigned to more effective treatments (e.g. fingolimod, natalizumab) whereas participants with low pretreatment MS activity are likely to be treated with less powerful drugs (e.g. interferon

beta). Baseline confounding by indication is likely the most frequent confounder in NRSIs. A cohort study directly comparing DMTs for MS should control for age, sex, MS duration, relapses, EDSS score and MRI activity measured before the start of DMT, because these are prognostic for the outcomes 'disability worsening' and 'relapse' and are also likely to influence treatment choice.

- Confounding by indication when shifting from a previous treatment to the treatment of interest (pre-intervention confounder, see the considerations above).
- Duration of follow-up from the start of the treatment of interest or the control treatment (confounder during the intervention). In some NRSIs, participants may be observed for different follow-up periods due to differences in drug licensing and availability across different geographical and historical cohorts (Trojano 2017). Such a difference in follow-up duration may be a confounder, particularly in medium- and long-term outcomes.

Adverse events

We assessed characteristics associated with the monitoring and reporting of adverse events, considering specific factors that may have had a large influence on adverse event data. We evaluated methods of monitoring and detecting adverse events in each primary study in order to assess if the researchers:

- actively monitored for adverse events, or if they simply provided spontaneous reporting of adverse events; and
- defined adverse events according to an accepted international classification.

We report this information in Table 1.

Measures of treatment effect

For dichotomous outcomes, we reported risk ratio (RR) and 95% confidence intervals (CIs). For continuous outcomes, we calculated mean difference (MD), or standardised mean difference (SMD) if the same continuous outcome was measured with different metrics, and 95% CIs. We back-calculated any results that were generated with a SMD based on scales that most closely reflect the outcome measure of interest in the review, as listed under important outcomes.

Unit of analysis issues

For multi-armed trials, the intervention groups of interest were those that could be included in a pairwise comparison of intervention groups, which, if investigated alone, would have met the criteria for including studies in the review. For example, when we identified a study comparing AZA versus glatiramer acetate versus AZA plus glatiramer acetate, only one comparison (AZA versus glatiramer acetate) was used, since it addressed the review objective. However, if the study compared AZA versus glatiramer versus fingolimod, all pairwise comparisons of interventions would be relevant to the review. In this scenario, we treated multi-armed studies as multiple independent two-arm studies assessed in separate comparisons.

We determined estimates of participants with adverse events and treatment discontinuations for adverse events by taking as denominator the number of participants who took at least one dose of the treatment or control.

Dealing with missing data

We used data that reflected the intention-to-treat analysis for each included outcome (both continuous and dichotomous), other than for adverse events. We performed primary analysis using the number of participants experiencing the event in relation to the number of randomised participants. In the case of participants with missing data, we performed primary analysis without any imputation. For adverse events, we used data from participants who received at least one dose of the study medication.

Assessment of heterogeneity

To evaluate clinical heterogeneity within treatment comparisons, we assessed differences in types of MS, types of interventions and study duration. To evaluate methodological heterogeneity, we evaluated the study design, variation in outcome measures, and risk of bias. We assessed the presence of statistical heterogeneity using Chi^2 and I^2 statistics (Higgins 2003). For the latter, we used the following ranges as a rough guide to interpreting statistical heterogeneity: 0% to 40% might not be important; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; 75% to 100% is considerable heterogeneity (Deeks 2020). In the latter case, we explored possible explanations for this considerable heterogeneity through subgroup and sensitivity analysis.

Assessment of reporting biases

We retrieved fewer than 10 RCTs; therefore, we were not able to evaluate the possibility of reporting bias for the primary outcomes by means of contour-enhanced funnel plots (Peters 2008). For our assessment of the risk of selective outcome reporting in the included studies, we compared published results against study protocols.

Data synthesis

We pooled the results from RCTs in pairwise meta-analysis while results from NRSIs were summarised narratively using the Synthesis Without Meta-analysis (SWiM) methodology (Campbell 2020).

For dichotomous outcomes, we estimated the between-study variance by means of the Mantel-Haenszel method. We used a random-effects model, assuming a certain degree of heterogeneity amongst studies and that studies were not always estimating the same intervention effect. We also assumed that such effects follow a normal distribution across studies (DerSimonian 1986). We used the inverse variance method and the random-effects model to synthesise continuous outcome measures.

In order to obtain an estimate of the harms of azathioprine versus no treatment, we pooled data on adverse events from all RCTs. Unlike for measures of benefit, when addressing harms, there is no reason to think that people with RRMS would experience different adverse events than those with PPMS.

Synthesis without meta-analysis

When we judged NRSIs as being at critical risk of bias using ROBINS-I, we excluded data from that NRSI from analysis.

We identified substantial clinical, methodological and statistical heterogeneity across NRSIs, which prevented pooling of data.

Therefore, we described such results narratively in a structured, tabulated format, following the *Cochrane Handbook for Systematic Reviews of Interventions* (McKenzie 2020) and the SWiM guidance (Campbell 2020).

Subgroup analysis and investigation of heterogeneity

It was not possible for us to conduct a subgroup analysis for the primary outcomes based on the presence of active or non-active MS as effect modifiers and possible sources of heterogeneity because no study presented results according to this classification.

Sensitivity analysis

In the protocol (Nonino 2021), we had planned sensitivity analysis for primary and secondary outcomes after excluding trials that we judged to be at high risk of bias. However, we judged all included trials to be at high risk of bias or at unclear risk because of the lack of details on concealment of allocation and selective reporting in the trial reports. As such, we did not perform any sensitivity analysis.

Summary of findings and assessment of the certainty of the evidence

In the summary of findings tables, we prioritised the critical and important clinical outcomes as listed in Types of outcome measures. Two review authors (EB, FN) assessed the certainty of evidence for each outcome, considering risk of bias, indirectness, inconsistency, imprecision of effect estimates and risk of publication bias. We resolved any disagreements by discussion. Where necessary, we consulted a third review author (GF). We provide our reasons for downgrading the certainty in the estimates of studies in the footnotes of the tables. Certainty in the estimates was appraised using GRADEpro GDT software (GRADEproGDT) as high, moderate, low, or very low. RCTs start at high certainty and can be downgraded up to three levels. For non-randomised studies, we started our certainty assessment at the level of high certainty, and downgraded according to GRADE methodology.

We reported the following critical and important outcomes in the summary of findings tables.

- Number of participants with disability worsening
- Number of participants with recurrence of relapses
- Number of participants with SAEs
- Number of participants reporting impairment in quality of life (mental score)
- Number of participants experiencing short-term adverse events (gastrointestinal disorders)
- Number of participants experiencing long-term adverse events (neoplasms)
- Overall number of deaths

We present four key SoF tables, addressing the following comparisons for people with RMS or PMS.

- AZA as a first-choice treatment compared with other DMTs (interferon beta) for RMS
- AZA when switching from a different DMT compared with other DMTs (interferon beta) for RMS
- AZA as a first-choice treatment compared with other DMTs (interferon beta) for PMS
- AZA when switching from a different DMT compared with other DMTs (interferon beta) for PMS

We included additional important outcomes for the above comparisons, as well as data for the above comparisons for DMTs other than interferon beta and the following additional comparisons in the 'Additional tables' section:

- AZA as a first-choice treatment compared with placebo for RMS;
- AZA when switching from a different DMT compared with placebo for RMS;
- AZA as a first-choice treatment compared with placebo for PMS; and
- AZA when switching from a different DMT compared with placebo for PMS.

We also included summaries of findings for NRSIs for all outcomes in the 'Additional tables' section.

RESULTS

Description of studies

We provide a description of studies in Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification; Characteristics of ongoing studies.

Results of the search

The search (18 March 2022, updated in August 2023) yielded 2056 reports: 2043 from electronic searches and 13 from searching other sources (Figure 1). After duplicates were removed, 1714 reports remained, of which we excluded 1640 based on title or abstract. Of the remaining 74 reports, we were unable to obtain full texts for 19 (see Studies awaiting classification) and we excluded a further 28 reports (corresponding to 26 studies) because the inclusion criteria were not met (see Excluded studies). Three reports were of ongoing studies (see Ongoing studies).

Figure 1. PRISMA flow diagram for the study selection process

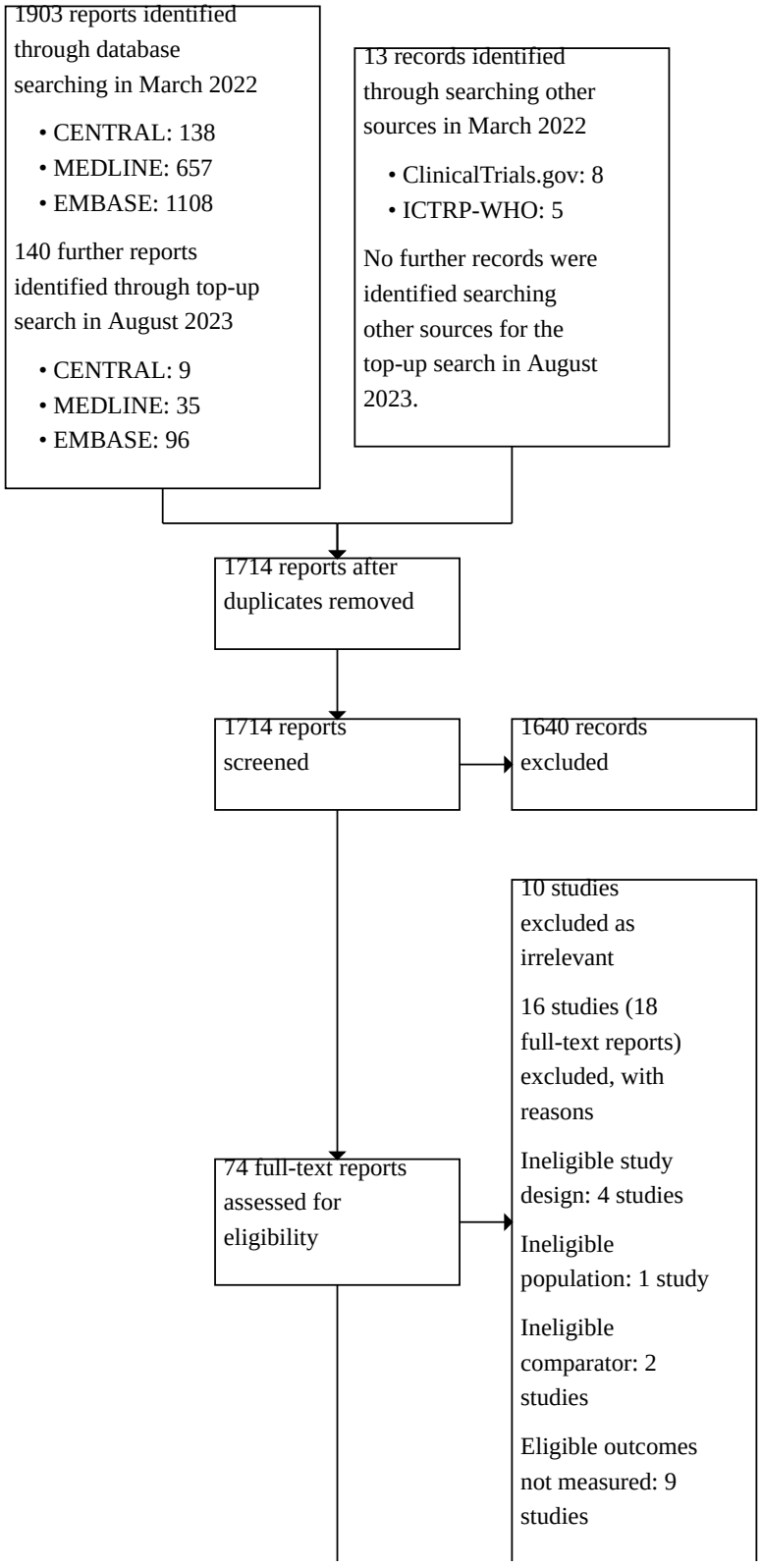
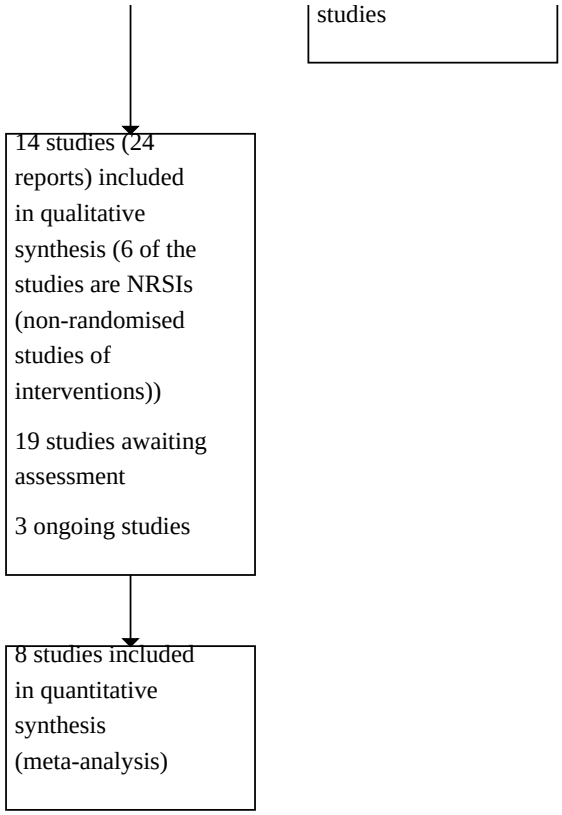


Figure 1. (Continued)



We included 24 full-text reports, which reported on 14 studies: 18 reports described eight randomised control trials (RCTs) (British and Dutch 1988; Ellison 1989; Etemadifar 2007; Goodkin 1991; Havrdova 2009; Kappos 1988; Massacesi 2014; Milanese 1993) and six reports described non-randomized studies of interventions (NRSIs) (Amato 1993; Confavreux 1996; Kappos 1990; Milanese 2001; Putzki 2006; Swinburn 1973).

Through a top-up search conducted on 9 August 2023, we identified 140 further records (35 records in MEDLINE, 96 in Embase, 9 in CENTRAL), three of which were duplicates. We screened the 137 titles or abstracts and found that none of them were potentially eligible for full-text review or inclusion.

Included studies

We included 14 studies that involved 2105 participants. The studies were described in 23 reports. See Figure 1. Three RCTs were the subject of additional reports or later follow-up studies (British and Dutch 1988; Havrdova 2009; Kappos 1988), while one RCT, Milanese 1993, was the subject of a preliminary report.

For detailed information on demographic and clinical features of the population included in the studies, please refer to the Characteristics of included studies. We also provide an overview of the included studies for RCTs in Table 2 and NRSIs in Table 3, and a summary of key features of the studies below.

Randomised controlled trials

We included eight randomised-controlled trials (RCTs). Six were double-blind (British and Dutch 1988; Ellison 1989; Goodkin 1991; Havrdova 2009; Kappos 1988; Milanese 1993); two were single-blind with concealed assessment of the outcomes (Etemadifar 2007; Massacesi 2014).

Participants

The eight RCTs involved 1076 participants. Four RCTs included only participants with RRMS (Etemadifar 2007; Goodkin 1991; Havrdova 2009; Massacesi 2014), one included only participants with progressive MS (Ellison 1989) and the remaining three RCTs included a mixture of participants with relapsing-remitting (48 to 67%) or progressive MS (British and Dutch 1988; Kappos 1988; Milanese 1993).

The mean age of participants at entry into the study ranged from 27 (Etemadifar 2007) to 39 (British and Dutch 1988) years in the azathioprine group, and from 28 (Etemadifar 2007) to 38 years (British and Dutch 1988) in the control group. In three studies (Ellison 1989; Goodkin 1991; Milanese 1993), the mean age of participants was not reported. Instead, the authors reported mean age at disease onset and duration of the condition. In these RCTs, the mean age at MS onset amongst participants treated with azathioprine ranged from 29.41 (SD 8.52) (Goodkin 1991) to 30.7 (± 10.5) (Ellison 1989) years, while mean age at MS onset ranged from 29.6 (SD 8.6) (Milanese 1993) to 33.4 (± 9.5) (Ellison 1989) years amongst participants not treated with azathioprine.

Regarding sex/gender, the proportion of female-reported participants was higher in six RCTs (British and Dutch 1988; Etemadifar 2007; Goodkin 1991; Havrdova 2009; Kappos 1988; Massacesi 2014), ranging from 58% (British and Dutch 1988) to 83% (Havrdova 2009). The proportion of participants reported as female was lower than those reported as male in one RCT (Ellison 1989). Sex/gender was not reported in Milanese 1993, while in the remaining seven RCTs there were a total of 671 female and 365 male participants, giving an overall mean female-to-male ratio of 1.8.

The RCTs were conducted in seven different countries: the UK (300 participants, British and Dutch 1988), Germany (194 participants, Kappos 1988), Italy (190 participants (Massacesi 2014; Milanese 1993), the USA (126 participants, Ellison 1989; Goodkin 1991), the Czech Republic (118 participants, Havrdova 2009), Iran (94 participants, Etemadifar 2007) and the Netherlands (54 participants, British and Dutch 1988).

Setting

Studies were conducted in a single hospital centre (Ellison 1989; Etemadifar 2007; Goodkin 1991; Milanese 1993) or multiple hospital centres (British and Dutch 1988; Kappos 1988; Massacesi 2014). One study did not provide information about its setting (Havrdova 2009).

Interventions

All studies included an azathioprine intervention, ranging from 2 to 4.4 mg/kg/day. Havrdova 2009 combined this with a weekly dose of interferon beta-1a (30 µg).

Comparators

Comparators were placebo (British and Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993), cyclosporine A drinking solution (5 mg/kg/day) (Kappos 1988) or various interferon-beta regimens (Etemadifar 2007; Havrdova 2009; Massacesi 2014).

Outcomes

All eight RCTs measured disability progression and relapse rate (British and Dutch 1988), with the addition of adverse events (Ellison 1989; Etemadifar 2007; Havrdova 2009; Kappos 1988; Milanese 1993), as well as MRI outcomes (Goodkin 1991; Massacesi 2014). For further details of the measures used, please see Characteristics of included studies, and see Table 1 for information on definitions of adverse events.

Funding

Four studies received some funding or support from commercial interests (Goodkin 1991; Havrdova 2009; Kappos 1988; Massacesi 2014). Two did not provide this information (Etemadifar 2007; Milanese 1993), and the remainder were funded by governmental institutions or private philanthropic foundations (British and Dutch 1988; Ellison 1989).

Non-randomised studies of intervention

We included six controlled non-randomised studies of intervention (NRSIs). The study designs included prospective parallel cohort (Milanese 2001), retrospective controlled cohort (Amato 1993; Putzki 2006) and prospective controlled cohort (Swinburn 1973), as well as case-control (Confavreux 1996) and retrospective matched-pairs controlled (Kappos 1990).

Participants

The six NRSIs involved 1029 participants. Two NRSIs included only participants with RRMS (Milanese 2001; Swinburn 1973). The remaining four studies included mixed populations of participants with RRMS or PMS (Amato 1993; Confavreux 1996; Kappos 1990; Putzki 2006), all of which included more than 50% of participants with RRMS.

Two studies investigated the potential determinants of cancer in people with MS that had received treatment with azathioprine (Confavreux 1996; Putzki 2006).

In three NRSIs (Milanese 2001; Putzki 2006; Swinburn 1973), the mean age of participants in the azathioprine group ranged from 31.2 years (Milanese 2001) to 40.5 years (Putzki 2006), and from 38 years (Milanese 2001) to 42.8 years (Putzki 2006) in the control group. Two non-randomised studies reported mean age in years at MS onset, ranging from 27.94 (Amato 1993) to 34.5 years (Confavreux 1996) in the azathioprine group, and from 30.7 (Amato 1993) to 33.7 years (Confavreux 1996) in the control group. One non-randomised study reported the average age at study entry (33.4 to 38.9 years) for certain groups of participants (Kappos 1990).

Regarding sex/gender, the proportion of female participants was higher in 4 NRSIs (Amato 1993; Confavreux 1996; Kappos 1990; Milanese 2001), ranging from 57% (Confavreux 1996) to 75% (Milanese 2001). The proportion of participants reported as female (33%) was lower than those reported as male in one NRSI (Putzki 2006). One NRSI included only male participants (Swinburn 1973). In total, 639 female participants were included in the six NRSIs, representing 62% of the 1029 participants included overall, giving an overall female-to-male ratio of 1.6.

The NRSIs were all conducted in Europe: Italy (486 participants; Amato 1993; Milanese 2001); Germany (401 participants; Kappos 1990; Putzki 2006); France (92 participants, Confavreux 1996) and the UK (50 participants, Swinburn 1973).

Setting

Two studies did not explicitly state their setting (Milanese 2001; Swinburn 1973). The others originated from single hospital centres (Amato 1993; Confavreux 1996; Kappos 1990; Putzki 2006).

Intervention

The intervention in all cases was azathioprine. Two of the studies did not specify the dosage (Confavreux 1996; Milanese 2001). In two studies, azathioprine was administered at 2 to 2.5 mg/kg body weight (Kappos 1990; Swinburn 1973). The two remaining studies expressed the administered dose of azathioprine in different ways: mean daily dose 110.51 mg (Amato 1993) and median overall cumulative dosage 108 g (range 2 to 1080 g) (Putzki 2006).

Comparators

Comparators included placebo (Swinburn 1973), no treatment with azathioprine (Amato 1993; Confavreux 1996), treatment with any immunosuppressive agent (Kappos 1990; Putzki 2006). In one study, the comparator could be either interferon beta-1b or no treatment, according to patient's choice (Milanese 2001).

Outcomes

Outcomes in three NRSIs were malignancy and adverse events (Amato 1993), risk of cancer related to azathioprine exposure (Confavreux 1996), and malignancy and mortality (Putzki 2006). The outcomes of the other studies included disability progression, relapses, and physical and quality of life scores (Kappos 1990; Milanese 2001; Swinburn 1973). See Table 1 for information on adverse events definitions.

Funding

Funding came from government agencies and private philanthropic foundations (Confavreux 1996; Kappos 1990; Swinburn 1973) or no information about funding was provided (Amato 1993; Milanese 2001; Putzki 2006).

Excluded studies

We excluded 28 reports from 26 studies, 10 because they were clearly irrelevant. Of the remaining 16 studies, we found that 4 studies had an ineligible study design, including being uncontrolled (Markovic-Plese 2003), or having too short a treatment duration (Cendrowski 1971) or follow-up (Patzold 1978; Ravnborg 2009). We excluded Lhermitte 1984 for having an ineligible population as it included people less than 18 years old. We excluded two studies due to ineligible comparators as they used azathioprine with combination treatments that were not present in all arms (Mertin 1982; Ring 1974). We excluded nine studies because they did not measure any of our outcomes of interest (Braun Hashemi 2006; Caputo 1987; Cavazzuti 1997; Ellison 1984; Patzold 1982; Rosen 1979; Steck 1990 Zeeberg 1985; Zeeberg 1986). See Characteristics of excluded studies for further information.

Studies awaiting classification

We were unable to determine eligibility for 19 reports, because the full text or the abstract was unavailable or did not provide sufficient information to determine eligibility (Aimard 1978; Ciesielski 1974; Confavreux 1980; Danielczyk 1973; Ellison 1981; Frick 1971; Frick 1974a; Frick 1974b; Frick 1977; Gentile 1972; Ghezzi 1989; Göpel 1972; Handouk 2009; Hervet 1974; Hitzchke 1979; Lhermitte 1984; Schluep 1991; Wilkerson 1975; Yankov 1980). Many of these appear to be observational reports in local hospital journals that are no longer active or otherwise do not provide means to access them. Ghezzi 1989 was included in a previous Cochrane review on this subject (Casetta 2007), but we were unable to obtain access, and as such, we have not been able to evaluate it according to current methods. Please see the Characteristics of studies awaiting classification table for more information.

Ongoing studies

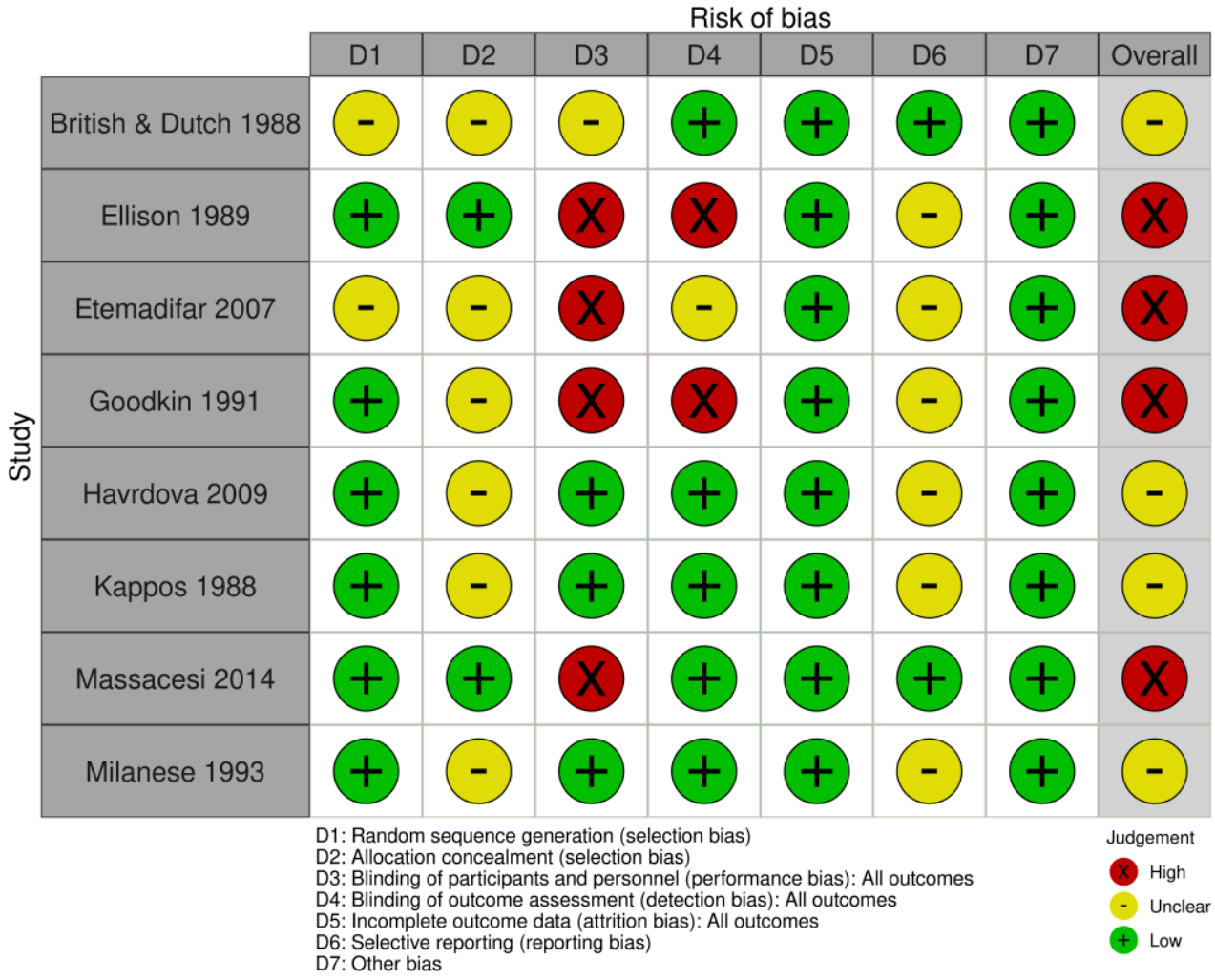
Three ongoing studies met our inclusion criteria. EUDRACT 2006-004937-13 is a multicentre RCT of azathioprine versus interferon beta in RRMS; however, there are no data and no further information is available. NCT03653273 is a randomised, controlled, open label, parallel-group study that aims to include 250 people with SPMS to compare DMT withdrawal versus continuation. NCT04106830 is a prospective cohort study investigating clinical and imaging outcomes in people with neuroinflammatory and demyelination diseases (including MS) who are being treated with a range of interventions, including azathioprine. Please see the Characteristics of ongoing studies table for more information.

Risk of bias in included studies

As outlined in Methods, we assessed RCTs using RoB1 according to Higgins 2017. We rated the overall risk of bias as high for four RCTs (Ellison 1989; Etemadifar 2007; Goodkin 1991 Massacesi 2014) and unclear for the other four RCTs (British and Dutch 1988; Havrdova 2009; Kappos 1988; Milanese 1993). Please see Figure 2 for a graph of our risk of bias assessments, Figure 3 for a risk of bias summary for the RCTs, and the Characteristics of included studies table for detailed information. Please see Table 4 for further information on the risk of bias in the NRSIs. We created the figures and tables using the robvis tool (McGuinness 2021).

Figure 2. Risk of bias graph: judgements about each risk of bias item presented as a percentage across all included randomised controlled trials

Figure 3. Risk of bias summary: judgements about each risk of bias item for every included randomised controlled trial



Allocation

We assessed six RCTs at low risk of bias for this domain as they used appropriate methods for their random sequence generation (Ellison 1989; Goodkin 1991; Havrdova 2009; Kappos 1988; Massacesi 2014; Milanese 1993). The other two RCTs were unclear because the information about the method was not provided (British and Dutch 1988) or because the randomisation method was unclear (Etemadifar 2007).

We assessed Ellison 1989 and Massacesi 2014 to be at low risk of bias for allocation concealment as the allocation sequence was adequately concealed. We assessed the rest of the RCTs as having unclear risk of bias due to no information being provided (British and Dutch 1988; Etemadifar 2007; Goodkin 1991; Havrdova 2009; Kappos 1988; Milanese 1993).

Blinding

We assessed Havrdova 2009, Kappos 1988 and Milanese 1993 as low risk of bias in this domain, as there was blinding of participants and personnel. We assessed British and Dutch 1988 as having an unclear risk of bias in this domain because the prescribing physician was non-masked. In Ellison 1989, Etemadifar 2007 and Goodkin 1991, there were detections of treatment groups by participants, healthcare professionals or trial personnel, and as such we judged them to be at high risk of bias in this domain, along with Massacesi 2014 where participants and treating neurologists were aware of treatment.

For blinding of outcome assessment (detection bias), we assessed Ellison 1989 as being at high risk of bias as a high proportion of assessing physicians correctly guessed the treatment allocation. Similarly, we considered the unblinding of treating physicians and the high number of participants that correctly guessed their treatment allocation as indicating a high risk of bias for Goodkin 1991. We judged Etemadifar 2007 to have an unclear risk of bias since it is possible that knowledge of treatment allocation may have biased participants' reporting of "subjective" outcomes. We assessed the remaining five RCTs as having a low risk of bias for

this domain, as the assessor was blinded (British and Dutch 1988; Havrdova 2009; Kappos 1988; Massacesi 2014; Milanese 1993).

Incomplete outcome data

We judged all the RCTs to be at low risk of bias in this domain, as information on loss to follow-up was provided, and proportions and causes of loss were similar between arms within studies.

Selective reporting

We assessed two RCTs as having a low risk of bias for selective reporting due to the reporting of prespecified analyses and outcomes (British and Dutch 1988) or having a pre-existing protocol (Massacesi 2014). Due to the lack of a protocol, we judged all other RCTs as being of unclear risk of bias for this domain (Ellison 1989; Etemadifar 2007; Goodkin 1991; Havrdova 2009; Kappos 1988; Milanese 1993).

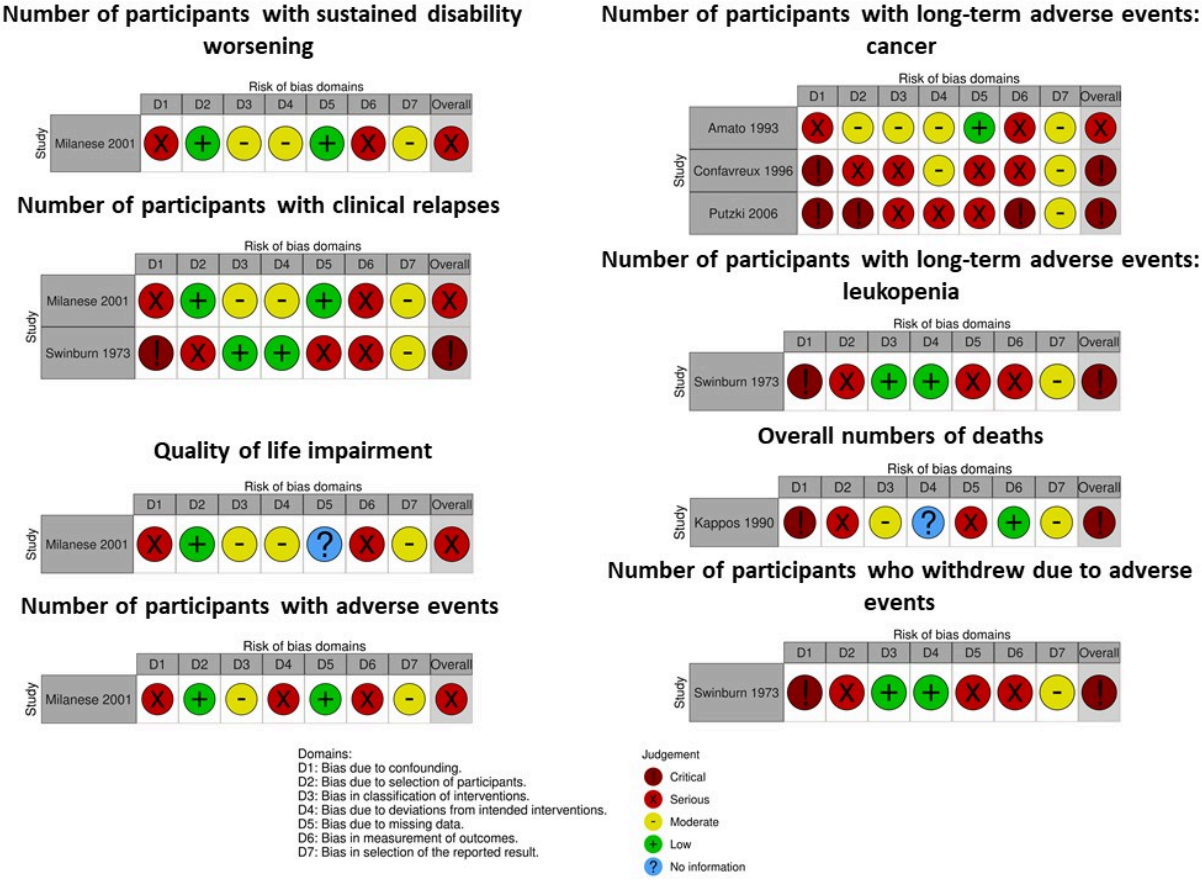
Other potential sources of bias

The RCTs appeared to be free of other sources of bias.

Risk of bias in non-randomised studies of interventions

We assessed the NRSIs using the ROBINS-I risk of bias tool (Sterne 2016). See Figure 4 for a summary and Table 4 for the full risk of bias tables for NRSIs, including support for our judgements.

Figure 4. Risk of bias (ROBINS-I) assessments for non-randomised studies of interventions *Abbreviations*
ROBINS-I: Risk Of Bias In Non-randomised Studies - of Interventions



Overall bias

We rated the overall risk of bias in Milanese 2001 as serious for the outcomes concerning disability worsening, clinical relapses, quality of life and adverse events. We rated Swinburn 1973 as having an overall critical risk of bias for outcomes concerning clinical relapses, long-term adverse events (leukopenia) and withdrawal due to adverse events. For the outcome of cancer as a long-term adverse event, we assessed the overall risk of bias as serious for Amato 1993 and critical for Confavreux 1996 and Putzki 2006. For mortality, we rated overall bias as critical for Kappos 1990. See Figure 4).

Bias due to confounding

Due to the fact that intervention allocation in Milanese 2001 was based on participant preference, we judged the risk of bias due to confounding to be serious for the outcomes 'number of participants with sustained disability worsening', 'number of participants with clinical relapses', 'mean change in quality of life score' and 'number of participants with adverse events'. Due to a lack of adjustment for potential confounders, we assessed Confavreux 1996 and Putzki 2006 as critical and Amato 1993 as serious for risk of bias for the outcome 'number of participants with long-term adverse events - cancer', and Swinburn 1973 as critical for outcomes concerning numbers of participants 'with clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due AEs'. Kappos

1990 was also rated as critical for 'overall number of deaths' as there was no information on the distribution of confounding factors amongst the included participants - only counts were available - and matched pairs were not maintained.

Bias in selection of participants into the study

For the outcomes 'number of participants with sustained disability worsening', 'number of participants with clinical relapses' and 'mean change in quality of life score', we judged the risk of bias as low for this domain in Milanese 2001. We judged the risk of bias in participant selection for long-term cancerous adverse events as critical for Putzki 2006, since only those self-selected patients responding to the questionnaire were included; serious for Confavreux 1996, as the start of follow-up and start of intervention were unlikely to coincide for most participants; and moderate for Amato 1993 because authors used appropriate methods to adjust for this bias. The risk of bias in participant selection was serious for Swinburn 1973 for outcomes concerning numbers of participants with 'clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due to AEs', since the analysis was based on participant characteristics (adverse events) observed after the start of intervention. As the start of follow-up and start of intervention do not coincide for most participants in Kappos 1990, we rated this study at serious risk of bias for the outcome 'overall number of deaths'.

Bias in classification of interventions

We rated Milanese 2001 as having moderate risk of bias in classification of interventions, as some aspects of assignment were determined retrospectively, for the outcomes 'number of participants with sustained disability worsening', 'number of participants with clinical relapses' and 'mean change in quality of life score', as well as for the 'number of participants with adverse events'. For the same reason, we judged Amato 1993 as being at moderate risk of bias for the outcome 'number of participants with long-term adverse events - cancer', while we rated Confavreux 1996 and Putzki 2006 as serious for risk of bias for this outcome due to a lack of clarity regarding interventions at the individual level. We rated Kappos 1990 as being at moderate risk of bias for the outcome 'overall number of deaths'. We rated Swinburn 1973 as low for this domain as the assignment was prospective, for the outcomes concerning numbers of participants 'with clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due to AEs'.

Bias due to deviations from intended interventions

We judged the risk of bias due to deviations from intended interventions as moderate for Milanese 2001 for the outcomes 'numbers of participants with sustained disability worsening', 'number of participants with clinical relapses' and 'mean change in quality of life score' due to a lack of information provided on deviations or co-interventions. We judged the risk of bias due to deviations from intended interventions as serious for the outcome 'number of participants with adverse events'. Regarding the 'number of participants with long-term cancerous adverse events', we judged Putzki 2006 as at serious risk of bias for this domain, and Amato 1993 and Confavreux 1996 as at moderate risk of bias for this domain. In Swinburn 1973, deviations were only those expected in usual practice, so we assessed it at low risk of bias for outcomes concerning numbers of participants 'with clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due to AEs'. There was no information provided on potential deviations from intended interventions for the outcome 'overall number of deaths' in Kappos 1990.

Bias due to missing data

We judged the risk of bias due to missing data as low for the outcomes 'number of participants with sustained disability worsening', 'number of participants with clinical relapses' and 'number of participants with adverse events' in Milanese 2001. For the quality of life outcome, the numbers of participants who did not complete the QoL questionnaire and number of non-completed items was not reported by Milanese 2001. We rated two studies as being at serious risk of bias for the outcome 'number of participants with long term adverse events - cancer' as it was not possible to know if all data were collected (Confavreux 1996; Putzki 2006), while we rated Amato 1993 at low risk of bias for this outcome as all participants were included in the analysis. We judged the risk of bias in Kappos 1990 as serious as it was not clear which participants were included in the 10-year follow-up. We rated Swinburn 1973 as having a serious risk of bias for outcomes concerning numbers of participants 'with clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due to AEs', given the lack of information about missing data or evidence of how it was dealt with.

Bias in measurement of outcomes

Due to a lack of blinding in outcome assessment, we judged the risk of bias for this domain as serious for the outcomes 'number of participants with sustained disability worsening', 'number of participants with clinical relapses', 'mean change in QoL score' and 'number of participants with serious adverse events' for Milanese 2001. We rated Swinburn 1973 as serious for outcomes concerning numbers of participants 'with clinical relapses', 'with long-term adverse events (leukopenia)' and 'who withdrew due to AEs'. For the outcome 'number of participants with long term adverse events - cancer', we rated Amato 1993 and Confavreux 1996 as being at serious risk of bias for lack of blinding in outcome assessment, and Putzki 2006 as critical because those participants not reporting neoplasm were not checked. We rated the risk of bias in Kappos 1990 as low for 'overall number of deaths' as the methods of outcome assessment were comparable across intervention groups.

Bias in selection of the reported result

We judged all outcomes for all NRSIs as being at moderate risk of bias in selection of the reported result due to none of these studies having an available protocol.

Effects of interventions

See: **Summary of findings 1** Azathioprine as a first-choice treatment versus other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis; **Summary of findings 2** Azathioprine when switching from a different disease-modifying therapy versus other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis; **Summary of findings 3** Azathioprine as a first-choice treatment versus other disease-modifying therapies (interferon beta) for progressive multiple sclerosis; **Summary of findings 4** Azathioprine when switching from a different disease-modifying therapy versus other disease-modifying therapies (interferon beta) for progressive multiple sclerosis

See Summary of findings 1; Summary of findings 2; Summary of findings 3; Summary of findings 4 for results from RCTs for our priority outcomes: disability, relapse, serious adverse events, quality-of-life impairment, long-term adverse events and mortality. Results from non-prioritised outcomes can be found in Table 5; Table 6; Table 7; Table 8; Table 9. See Table 9 for a summary of key findings for the non-randomised studies.

Randomised controlled trials

Azathioprine as a first-choice treatment compared with other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis

In Summary of findings 1, we provide a summary of the effect estimates for azathioprine as a first-choice treatment compared with interferon beta for relapsing MS for the critical and important outcomes specified in the Summary of findings and assessment of the certainty of the evidence section for RCTs.

Two RCTs compared azathioprine to interferon beta (Etemadifar 2007; Massacesi 2014).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability progression over two years compared to interferon beta (RR 0.19, 95% CI 0.02 to 1.58; 1 RCT, 148 participants; very low-certainty evidence); the absolute effect was 56 fewer per 1000 (95% CI 68 fewer to 40 more) experiencing disability worsening over two years with azathioprine compared to interferon beta (Analysis 1.1).

Relapse

Azathioprine may result in a possible decrease in the number of people with relapses over one to two years compared to interferon beta (RR 0.61, 95% CI 0.43 to 0.86; 2 RCTs, 242 participants; low-certainty evidence); the absolute effect was 177 fewer per 1000 (95% CI 259 fewer to 64 fewer) experiencing relapse over two years with azathioprine compared to interferon beta (Analysis 1.2).

Serious adverse events

The evidence suggests that azathioprine may result in a possible increase in the number of people with serious adverse events over two years compared to interferon beta (RR 6.64, 95% CI 0.35 to 126.27; 1 RCT, 148 participants; low-certainty evidence); the absolute effect was not estimable in GRADEpro due to the width of the CIs (Analysis 1.3).

Important outcomes (in priority order)

Short-term adverse events (gastrointestinal disorders)

Azathioprine may result in a possible increase in the number of people with nausea or vomiting over two years compared to interferon beta (RR 5.30, 95% CI 0.15 to 185.57; 2 RCTs, 242 participants; very low-certainty evidence); the absolute effect was 108 more per 1000 (95% CI 21 fewer to 4.653 more) experiencing nausea or vomiting over two years with azathioprine compared to interferon beta (Analysis 1.4).

Neither study included in this comparison measured our other important outcomes of interest: quality-of-life impairment (mental score), long-term adverse events (neoplasms) and mortality.

Additional important outcomes

See Table 5 for summary of finding tables for other secondary outcomes.

Other short-term adverse events (hypersensitivity reactions)

Azathioprine may result in a possible increase in the number of people with hypersensitivity reactions over two years compared to interferon beta (RR 6.64, 95% CI 0.35 to 126.27; 1 RCT, 148 participants; low-certainty evidence); the absolute effect was not estimable in GRADEpro due to the width of the CIs (Analysis 1.5).

Other long-term adverse events

- **Influenza-like illness:** azathioprine may result in a possible reduction in the number of people with influenza-like illness over two years compared to interferon beta (RR 0.08, 95% CI 0.03 to 0.24; 2 RCTs, 242 participants; low-certainty evidence); the absolute effect was 317 fewer per 1000 (95% CI 334 fewer to 262 fewer) experiencing influenza-like illness over two years with azathioprine compared to interferon beta (Analysis 1.6).
- **Leukopenia:** azathioprine likely results in an increase in the number of people with leukopenia over two years compared to interferon beta (RR 2.07, 95% CI 1.09 to 3.91; 1 RCT, 148 participants; moderate-certainty evidence); the absolute effect was 163 more per 1000 (95% CI 14 more to 445 more) experiencing leukopenia over two years with azathioprine compared to interferon beta (Analysis 1.7).
- **Hepatobiliary disorders:** azathioprine may result in a possible decrease in the number of people with hepatobiliary disorders over two years compared to interferon beta (RR 0.56, 95% CI 0.31 to 1.03; 1 RCT, 148 participants; low-certainty evidence); the absolute effect was 134 fewer per 1000 (95% CI 211 fewer to 9 more) experiencing hepatobiliary disorders over two years with azathioprine compared to interferon beta (Analysis 1.8).

Annualised relapse rate (ARR)

Azathioprine may result in a possible decrease in the annualised relapse rate over two years compared to interferon beta (1 RCT, 150 participants; very low-certainty evidence); the absolute effect was MD 0.13 lower (95% CI 0.27 lower to 0.01 higher) numbers of relapses per person-year at risk (Analysis 1.9). Of note, the ARR calculation was based not on all 150 randomised participants, but only on 127 (azathioprine n = 62, interferon n = 65) who completed the 24-month follow-up, and we downgraded the certainty for risk of bias due to incomplete outcome data.

New or enlarging T2-weighted MRI lesions

Azathioprine may result in a possible decrease in the number of people with new or enlarged T2 weighted MRI lesions over two years compared to interferon beta (RR 0.88, 95% CI 0.57 to 1.37; 1 RCT, 122 participants; very low-certainty evidence); the absolute effect was 51 fewer per 1000 (95% CI 183 fewer to 158 more) experiencing new or enlarged T2 weighted lesions over two years with azathioprine compared to interferon beta (Analysis 1.10).

New gadolinium-enhancing T1-weighted MRI lesions

Azathioprine may result in a possible increase in the number of people with new gadolinium-enhancing T1-weighted MRI lesions over two years compared to interferon beta (RR 2.00, 95% CI 0.64 to 6.29; 1 RCT, 122 participants; very low-certainty evidence); the absolute effect was 66 more per 1000 (95% CI 24 fewer to 347

more) experiencing new gadolinium-enhancing T1-weighted MRI lesions overtwo years with azathioprine compared to interferon beta (Analysis 1.11).

Treatment discontinuation due to adverse events (AEs)

Azathioprine may result in a possible increase in the number of people discontinuing treatment due to AEs over one year compared to interferon beta (RR 1.81, 95% CI 0.83 to 3.94; 2 RCTs, 242 participants; very low-certainty evidence); the absolute effect was 61 more per 1000 (95% CI 13 fewer to 222 more) discontinuing due to adverse events over one year with azathioprine compared to interferon beta Analysis 1.12.

Neither study included in this comparison measured our additional important outcomes of interest: adverse events, quality-of-life impairment (physical score) and cognitive decline.

Azathioprine as a first-choice treatment or when switching from a different disease-modifying therapy compared with other disease-modifying therapies (interferon beta)

None of the included studies reported outcomes relevant to the following comparisons that were identified as core comparisons in the Summary of findings and assessment of the certainty of the evidence section of the review.

- Azathioprine when switching from a different disease-modifying therapy compared with other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis (Summary of findings 2).
- Azathioprine as a first-choice treatment compared with other disease-modifying therapies (interferon beta) for progressive multiple sclerosis (Summary of findings 3).
- Azathioprine when switching from a different disease-modifying therapy compared with other disease-modifying therapies (interferon beta) for progressive multiple sclerosis (Summary of findings 4).

Azathioprine as a first-choice treatment compared with other disease-modifying therapies (cyclosporine A) for relapsing multiple sclerosis

In Table 6, we provide a summary of the effect estimates for azathioprine as a first-choice treatment compared with cyclosporine A for relapsing MS for the priority outcomes specified in the Summary of findings and assessment of the certainty of the evidence section.

One RCT compared azathioprine to cyclosporine A (Kappos 1988).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability progression over two years compared to cyclosporine A (RR 0.98, 95% CI 0.59 to 1.63; 1 RCT, 194 participants; very-low certainty evidence); the absolute effect was 5 fewer per 1000 (95% CI 138 fewer to 383 more) experiencing disability worsening over two years with azathioprine compared to cyclosporine A (Analysis 2.1).

Relapse

The RCT did not measure this outcome.

Serious adverse events (SAEs)

Azathioprine may result in a possible decrease in the number of people with SAEs over two years compared to cyclosporine A (RR 0.34, 95% CI 0.01 to 8.25; 1 RCT, 194 participants; low certainty-evidence); the absolute effect was 7 fewer per 1000 (95% CI 10 fewer to 74 more) experiencing SAEs over two years with azathioprine compared to cyclosporine A (Analysis 2.2).

Important outcomes

Short-term adverse events (gastrointestinal disorders)

Azathioprine may result in no difference in the number of people with gastrointestinal disorders over two years compared to cyclosporine A (RR 1.00, 95% CI 0.78 to 1.29; 1 RCT, 194 participants; low-certainty evidence); the absolute effect was 0 fewer per 1000 (95% CI from 121 fewer to 160 more) experiencing gastrointestinal disorders over two years with azathioprine compared to cyclosporine A (Analysis 2.3).

The study included in this comparison did not measure our other important outcomes of interest: quality-of-life impairment (mental score), long-term adverse events (neoplasms) and mortality.

Additional important outcomes

Other short-term adverse events (hypersensitivity reactions)

Azathioprine may result in a possible decrease in the number of people with hypersensitivity reactions over two years compared to cyclosporine A (RR 0.84, 95% CI 0.36 to 1.92; 1 RCT, 194 participants; low-certainty evidence); the absolute effect was 18 fewer per 1000 (95% CI from 72 fewer to 103 more) experiencing hypersensitivity reactions over two years with azathioprine compared to cyclosporine A (Analysis 2.4).

Other long-term adverse events

- **Leukopenia:** azathioprine likely results in a large increase in the number of people with leukopenia over two years compared to cyclosporine A (RR 6.51, 95% CI 3.26 to 12.98; 1 RCT; 194 participants; moderate-certainty evidence); the absolute effect was 450 more per 1000 (95% CI 184 more to 978 more) experiencing leukopenia over two years with azathioprine compared to cyclosporine A (Analysis 2.5).
- **Hepatobiliary disorders:** azathioprine may result in a possible increase in the number of people with hepatobiliary disorders over two years compared to cyclosporine A (RR 1.40, 95% CI 0.90 to 2.19; 1 RCT, 194 participants; low-certainty evidence); the absolute effect was 98 more per 1000 (95% CI 24 fewer to 291 more) experiencing hepatobiliary disorders over two years with azathioprine compared to cyclosporine A (Analysis 2.6).
- **Infections:** azathioprine may result in a possible reduction in the number of people with infections over two years compared to cyclosporine A (RR 0.92, 95% CI 0.71 to 1.21; 1 RCT, 194 participants; low-certainty evidence); the absolute effect was 43 fewer per 1000 (95% CI from 157 fewer to 114 more) experiencing infections over two years with azathioprine compared to cyclosporine A (Analysis 2.7).
- **CNS disorders (paraesthesia):** azathioprine likely results in a slight reduction in the number of people with paraesthesia over two years compared to cyclosporine A (RR 0.22, 95% CI 0.10 to 0.48; 1 RCT, 194 participants; moderate-certainty evidence); the absolute effect was 255 fewer per 1000 (95% CI from 294

fewer to 170 fewer) experiencing paraesthesia over two years with azathioprine compared to cyclosporine A (Analysis 2.8).

- **Skin and subcutaneous tissue disorders:** azathioprine likely results in a slight reduction in the number of people with hypertrichosis over two years compared to cyclosporine A (RR 0.29, 95% CI 0.17 to 0.49; 1 RCT, 194 participants; moderate-certainty evidence); the absolute effect was 355 fewer per 1000 (95% CI from 415 fewer to 255 fewer) experiencing hypertrichosis over two years with azathioprine compared to cyclosporine A (Analysis 2.9).

Annualised relapse rate (ARR)

Azathioprine may result in a possible decrease in the ARR over two years compared to cyclosporine A (MD 0.18, 95% CI 0.10 to 0.26; 1 RCT, 194 participants; low-certainty evidence); the absolute effect was MD 0.18 higher (from 0.10 to 0.26 higher) numbers of relapses per person-year at risk over two years with azathioprine compared to cyclosporine A (Analysis 2.10).

Treatment discontinuation due to adverse events

Azathioprine may result in a possible increase in the number of people discontinuing treatment due to adverse events over two years compared to cyclosporine A (RR 1.17, 95% CI 0.44 to 3.09; 1 RCT, 194 participants, low certainty evidence); the absolute effect was 12 more per 1000 (95% CI from 40 fewer to 149 more) discontinuing due to AEs over two years with azathioprine compared to cyclosporine A (Analysis 2.11).

The study included in this comparison did not measure our other additional important outcomes of interest: adverse events, quality-of-life impairment (physical score), cognitive decline, new or enlarging T2-weighted MRI lesions and new gadolinium-enhancing T1-weighted MRI lesions.

Azathioprine as a first-choice treatment compared with placebo for relapsing multiple sclerosis

In Table 7, we provide a summary of the effect estimates for azathioprine as a first-choice treatment compared with placebo for relapsing MS for the outcomes specified in the Summary of findings and assessment of the certainty of the evidence section.

Three RCTs compared azathioprine to placebo (British and Dutch 1988; Goodkin 1991; Havrdova 2009).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability progression over two to three years compared to placebo, (RR 0.93, 95% CI 0.47 to 1.86; 2 RCTs, 177 participants; very low-certainty evidence); the absolute effect was 14 fewer per 1000 (95% CI 107 fewer to 174 more) experiencing disability worsening over two to three years with azathioprine compared to placebo (Analysis 3.1).

Relapse

Azathioprine may result in a possible decrease in the number of people with relapses over one to two years compared to placebo (RR 0.90, 95% CI 0.73 to 1.12; 2 RCTs, 177 participants; very low-certainty evidence); the absolute effect was 68 fewer per 1000 (95% CI 185 fewer to 83 more) experiencing relapse over one to two years with azathioprine compared to placebo (Analysis 3.2).

Serious adverse events (SAEs)

Azathioprine may result in a possible increase in the number of people with serious adverse events over two years compared to placebo (RR 2.07, 95% CI 0.19 to 22.20; 1 RCT, 118 participants; very low-certainty evidence); the absolute effect was 18 more per 1000 (95% CI 14 fewer to 353 more) experiencing serious adverse events over two years with azathioprine compared to placebo (Analysis 3.3).

Important outcomes

Quality-of-life impairment (mental score)

No RCT measured this outcome.

Short-term adverse events (gastrointestinal disorders)

Azathioprine likely results in an increase in the number of people with gastrointestinal adverse events over three years compared to placebo (RR 2.25, 95% CI 1.32 to 3.84; 1 RCT, 354 participants; moderate-certainty evidence); the absolute effects was 118 more per 1000 (95% CI 30 more to 268 more) experiencing gastrointestinal adverse events over three years with azathioprine compared to placebo (Analysis 3.4).

Long-term adverse events (neoplasms)

Azathioprine may result in a possible increase in the number of people with neoplasms at 14 years compared to placebo (RR 1.74, 95% CI 0.70 to 4.29; 1 RCT, 300 participants; very low-certainty evidence); the absolute effect was 34 more per 1000 (95% CI 14 fewer to 153 more) with neoplasms over 14 years with azathioprine compared to placebo (Analysis 3.5).

Mortality

- Azathioprine may result in a possible increase in the number of deaths at three years compared to placebo (RR 3.62, 95% CI 0.76 to 17.19; 1 RCT, 354 participants; low-certainty evidence); the absolute effect was 29 more per 1000 (95% CI 3 fewer to 180 more) deaths over three years with azathioprine compared to placebo (Analysis 3.6).
- Azathioprine may result in a possible decrease in the number of deaths at 14 years compared to placebo (RR 0.86, 95% CI 0.58 to 1.28; 1 RCT, 300 participants; very low-certainty evidence); the absolute effect was 37 fewer per 1000 (95% CI 111 fewer to 74 more) deaths over 14 years with azathioprine compared to placebo (Analysis 3.7).

Additional important outcomes

Other short-term adverse events (hypersensitivity reactions)

Azathioprine likely results in a slight increase in the number of people with hypersensitivity reactions over two years compared to placebo (RR 2.32, 95% CI 1.17 to 4.60; 2 RCTs, 472 participants; moderate-certainty evidence); the absolute effects was 60 more per 1000 (95% CI 8 more to 165 more) experiencing hypersensitivity reactions over two years with azathioprine compared to placebo (Analysis 3.8).

Other long-term adverse events

- **Leukopenia - blood and lymphatic system disorders:** azathioprine likely results in a large increase in the number of people with leukopenia over three years compared to placebo (RR 14.48, 95% CI 4.57 to 45.86; 1 RCT, 354 participants; moderate-certainty evidence); the absolute effect was 225 more per 1000 (95% CI 60 more to 748 more) experiencing leukopenia over three years with azathioprine compared to placebo (Analysis 3.9).
- **Hepatobiliary disorders:** azathioprine may result in a possible increase in the number of people with hepatobiliary disorders over two to three years compared to placebo (RR 1.83, 95% CI 0.37 to 9.08; 2 RCTs, 472 participants; very low-certainty evidence); the absolute effect was 35 more per 1000 (95% CI 26 fewer to 337 more) experiencing hepatobiliary disorders over two to three years with azathioprine compared to placebo (Analysis 3.10).
- **Infections:** azathioprine may result in a possible increase in the number of people with infections over two years compared to placebo (RR 1.06, 95% CI 0.85 to 1.33; 1 RCT, 118 participants; very low-certainty evidence); the absolute effect was 27 more per 1000 (95% CI 69 fewer to 151 more) experiencing infections over two years with azathioprine compared to placebo (Analysis 3.11).
- **Skin and subcutaneous tissue disorders:** azathioprine may result in a possible increase in the number of people with skin and subcutaneous tissue disorders over two years compared to placebo (RR 4.14, 95% CI 0.48 to 35.93; 1 RCT, 118 participants; very low-certainty evidence); the absolute effect was 52 more per 1000 (95% CI 9 fewer to 582 more) experiencing skin and subcutaneous tissue disorders over two years with azathioprine compared to placebo (Analysis 3.12).

Treatment discontinuation due to adverse events

Azathioprine may result in a possible increase in the number of people discontinuing treatment due to AEs over two to three years compared to placebo (RR 4.85, 95% CI 0.60 to 39.14; 3 RCTs, 526 participants; very low-certainty evidence); the absolute effect was 44 more per 1000 (95% CI 4 fewer to 433 more) discontinuing due to AEs over two to three years with azathioprine compared to placebo (Analysis 3.13).

The studies included in this comparison did not measure our other additional important outcomes of interest: adverse events, quality-of-life impairment (physical score), annualised relapse rate, cognitive decline, new or enlarging T2-weighted MRI lesions and new gadolinium-enhancing T1-weighted MRI lesions.

Azathioprine when switching from a different disease-modifying therapy compared with placebo for relapsing multiple sclerosis

None of the included studies reported outcomes relevant to this comparison.

Azathioprine as a first-choice treatment compared with placebo for progressive multiple sclerosis

In Table 8, we provide a summary of the effect estimates for azathioprine as a first-choice treatment compared with placebo for progressive MS for the outcomes specified in the Summary of findings and assessment of the certainty of the evidence section.

Two RCTs compared azathioprine to placebo in a study with people with progressive MS (Ellison 1989; Milanese 1993).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability progression over three years compared to placebo (RR 0.35, 95% CI 0.16 to 0.76; 1 RCT, 40 participants; very low-certainty evidence); the absolute effect was 495 fewer per 1000 (95% CI 640 fewer to 183 fewer) experiencing disability progression over three years with azathioprine compared to placebo (Analysis 4.1).

Relapse

Azathioprine may result in a possible decrease in the number of people with relapses over three years compared to placebo (RR 0.53, 95% CI 0.35 to 0.80; 2 RCTs, 107 participants; very low-certainty evidence); the absolute effect was 308 fewer per 1000 (95% CI 425 fewer to 124 fewer) experiencing relapses over three years with azathioprine compared to placebo (Analysis 4.2).

Serious adverse events

The two RCTs did not measure this outcome.

Important outcomes

Long-term adverse events (neoplasms)

Azathioprine may result in a possible increase in the number of people with neoplasms over three years compared to placebo (RR 1.10, 95% CI 0.07 to 16.80; 1 RCT, 65 participants; low-certainty evidence): the absolute effect was 3 more per 1000 (27 fewer to 465 more) with neoplasms over three years with azathioprine compared to placebo (Analysis 4.3).

Mortality

Azathioprine may result in a possible decrease in the number of deaths over three years compared to placebo (RR 0.55, 95% CI 0.05 to 5.75; 1 RCT, 65 participants; low-certainty evidence); the absolute effect was 26 fewer deaths per 1000 (95% CI 56 fewer to 279 more) over three years with azathioprine compared to placebo (Analysis 4.4).

Neither study included in this comparison measured our other important outcomes of interest: quality-of-life impairment (mental score) and short-term adverse events (gastrointestinal disorders).

Additional important outcomes

Other short-term adverse events (hypersensitivity reactions)

Azathioprine may result in a possible increase in the number of people with hypersensitivity reactions over two to three years compared to placebo (RR 7.66, 95% CI 0.41 to 142.55; 1 RCT, 65 participants; low-certainty evidence); the absolute effect was not estimable in GRADEpro due to the width of the CI (Analysis 4.5).

Other long-term adverse events

- **Leukopenia - blood and lymphatic system disorders:** azathioprine may result in a possible increase in the number of people with leukopenia over three years compared to placebo (RR 5.47, 95% CI 0.27 to 109.65; 1 RCT, 65 participants; low-

certainty evidence): the absolute effect was not estimable in GRADEpro due to the width of the CIs (Analysis 4.6).

- **Infections:** azathioprine may result in a possible increase in the number of people with infections over three years compared to placebo (RR 1.10, 95% CI 0.07 to 16.80; 1 RCT, 65 participants; low-certainty evidence); the absolute effect was 3 more per 1000 (95% CI 27 fewer to 465 more) experiencing infections over three years with azathioprine compared to placebo (Analysis 4.7).

Treatment discontinuation due to adverse events

Azathioprine may result in a possible increase in the number of people discontinuing treatment due to AEs over three years compared to placebo (RR 8.73, 95% CI 1.13 to 67.42; 2 RCTs, 105 participants; low-certainty of evidence); the absolute effect was not estimable in GRADEpro due to the width of the CIs (Analysis 4.8).

The studies included in this comparison did not measure our other additional important outcomes of interest: adverse events, quality-of-life impairment (physical score), annualised relapse rate, cognitive decline, new or enlarging T2-weighted MRI lesions and new gadolinium-enhancing T1-weighted MRI lesions.

Azathioprine when switching from a different disease-modifying therapy compared with placebo for progressive multiple sclerosis

None of the included studies reported outcomes relevant to this comparison.

Non-randomised studies of interventions

We assessed four of the non-randomised studies as having critical risk of bias (see Table 4), and we did not consider them further in the synthesis (Confavreux 1996; Kappos 1990; Putzki 2006; Swinburn 1973). In Table 9, we provide a summary of findings for all outcomes explored in the rest of the non-randomised studies of interventions (Amato 1993; Milanese 2001).

Azathioprine as a first-choice treatment compared with other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis

One non-randomised cohort study compared 32 participants with relapsing remitting MS who started their first treatment with azathioprine, interferon beta-1b or no treatment according to patient choice (Milanese 2001).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability worsening over one year compared with interferon beta 1-b (RR 0.36, 95% CI 0.02 to 8.03; 21 participants; very low-certainty evidence); the absolute effect was 58 fewer per 1000 (95% CI 89 fewer to 639 more); experiencing disability worsening over one year with azathioprine compared to interferon beta-1b (Analysis 5.1).

Relapse

Azathioprine may result in a possible decrease in the number of people with relapses over one year compared with interferon beta 1-b (RR 0.47, 95% CI 0.17 to 1.34; 21 participants; very low-certainty evidence); the absolute effect was 337 fewer per 1000 (95% CI 528 fewer to 216 more) experiencing relapses over one year with azathioprine compared to interferon beta-1b (Analysis 5.2).

Serious adverse events

The NRSI did not measure this outcome.

Important outcomes

Quality-of-life impairment (mental score)

Azathioprine may result in a possible increase in the mean difference in the change of the mental composite score over one year compared to interferon beta 1-b; the absolute effect was MD 27.29 higher (95% CI 16.25 higher to 38.33 higher; 21 participants; very low-certainty evidence) in the mental composite score over one year with azathioprine compared to interferon beta 1-b (Analysis 5.3).

The non-randomised study included in this comparison did not measure our other important outcomes of interest: short-term adverse events (gastrointestinal disorders), long-term adverse events (neoplasms) and mortality.

Additional important outcomes

Other short-term adverse events

Azathioprine may result in a possible decrease in the number of people with influenza-like illness over one year compared to interferon beta 1-b (RR 0.10 95% CI 0.01 to 1.59; 21 participants; very low-certainty evidence); the absolute effect was 409 fewer per 1000 (95% CI 405 fewer to 268 more) experiencing influenza-like illness over one year with azathioprine compared to interferon beta-1b (Analysis 5.4).

Quality-of-life impairment (physical score)

Azathioprine may result in a possible increase in the mean difference in the change of the physical composite score over one year compared to interferon beta 1-b; the absolute effect was MD 10.54 higher (95% CI 2.32 higher to 18.76 higher; 21 participants; very low-certainty evidence, Milanese 2001) in the physical composite score over one year with azathioprine compared to interferon beta-1b (Analysis 5.5).

The non-randomised study included in this comparison did not measure our other additional important outcomes of interest: other long-term adverse events, adverse events, annualised relapse rate, cognitive decline, new or enlarging T2-weighted MRI lesions, new gadolinium-enhancing T1-weighted MRI lesions and treatment discontinuation due to adverse events.

Azathioprine as a first-choice treatment compared with placebo or no treatment for relapsing multiple sclerosis

Milanese 2001 also compared people with relapsing remitting MS starting treatment with azathioprine (10 participants) versus no treatment (11 participants). Amato 1993 is a retrospective, parallel cohort study of 454 people with mostly relapsing forms of MS who were treated with azathioprine for at least three months (207 participants) or did not undergo treatment with azathioprine (247 participants).

Critical outcomes

Disability

Azathioprine may result in a possible decrease in the number of people with disability worsening over one year compared to no treatment (RR 0.20, 95% CI 0.01 to 3.70; very low-certainty evidence; Milanese 2001); the absolute effect was 160 fewer per 1000 (95% CI 198 fewer to 540 more) experiencing disability worsening over one year with azathioprine compared to no treatment (Analysis 5.6).

Relapse

Azathioprine may result in a possible decrease in the number of people with relapses after one year (RR 0.50, 95% CI 0.17 to 1.46; very low-certainty evidence; Milanese 2001); the absolute effect was 300 fewer per 1000 (95% CI 498 fewer to 276 more) experiencing relapses over one year with azathioprine compared to no treatment (Analysis 5.7).

Serious adverse events

The NRSIs did not measure this outcome.

Important outcomes

Quality-of-life impairment (mental score)

Azathioprine may result in a possible increase in the mean difference in the change of mental composite score over one year; the absolute effect was MD 14.88 higher (95% CI 0.04 higher to 29.72 higher; very low-certainty evidence; Milanese 2001) in the mental composite score over one year with azathioprine compared to no treatment (Analysis 5.8).

Long-term adverse events (neoplasms)

Azathioprine may result in a possible decrease in the number of people with neoplasms over 10 years compared to no treatment (age-adjusted RR 0.81, 95% CI 0.26 to 2.50; very low-certainty evidence; Amato 1993); the absolute effect was 5 fewer per 1000 (95% CI 21 fewer to 45 more) experiencing neoplasms over 10 years with azathioprine compared to no treatment (Analysis 5.9).

The non-randomised studies included in this comparison did not measure our other important outcomes of interest: short-term adverse events (gastrointestinal disorders) and mortality.

Additional important outcomes

Quality-of-life impairment (physical score)

Azathioprine may result in a possible increase in the mean difference in the change of the physical composite score over one year compared to no treatment; the absolute effect was MD 4.24 higher (95% CI -5.92 lower to 14.40 higher; very low-certainty evidence, Milanese 2001) in the physical composite score over one year with azathioprine compared to no treatment (Analysis 5.10).

The non-randomised study included in this comparison did not measure our other additional important outcomes of interest: other short-term adverse events, other long-term adverse events, adverse events, annualised relapse rate, cognitive decline, new or enlarging T2-weighted MRI lesions, new gadolinium-enhancing T1-weighted MRI lesions and treatment discontinuation due to adverse events.

DISCUSSION

In this review, we aimed to summarise available evidence for the benefits and harms of azathioprine for people with multiple sclerosis (MS), compared to other disease-modifying therapies (DMTs), as well as compared to placebo or no treatment. We evaluated the priority outcomes of disability worsening, relapse, serious adverse events, quality-of-life impairment (mental score), as well as short term (gastrointestinal disorders) and long term (neoplasms) adverse events, and mortality (see Effects of interventions). We also assessed additional outcomes relating to adverse events not otherwise covered in the priority outcomes, as well as cognitive impairment, discontinuation of treatment and magnetic resonance imaging (MRI) findings relating to lesions.

We included eight randomised control trials (RCTs) (1076 participants) and six non-randomised studies of interventions (NRSIs) (1029 participants). The included studies compared azathioprine with placebo, no treatment, various interferon beta regimens or cyclosporine A. Most outcomes were assessed at two to three years of follow-up, with only two outcomes (neoplasms and mortality) assessed at longer time points, meaning that the effects of azathioprine beyond two to three years for most outcomes is unknown.

Summary of main results

Evidence from RCTs

Azathioprine versus interferon beta

We prioritised comparisons of azathioprine to interferon beta when it was the 'first choice' treatment or when patients took another treatment first and switched to azathioprine.

In people with relapsing forms of MS, we found limited evidence from RCTs for azathioprine as a 'first choice' treatment compared to interferon beta (Summary of findings 1). Azathioprine as a first-choice treatment:

- may result in a possible decrease in the number of people with disability progression over two years compared to interferon beta (very low-certainty evidence);
- may result in a possible decrease in the number of people with relapses over one to two years compared to interferon beta (low-certainty evidence);
- may result in a possible increase in the number of people with serious adverse events (SAEs) over two years compared to interferon beta (low-certainty evidence); and
- may result in a possible increase in the number of people with the short-term adverse event 'gastrointestinal disorders' over two years compared to interferon beta (very low-certainty evidence).

We found no evidence comparing azathioprine:

- to other DMTs in RCTs that included people with progressive MS (Summary of findings 3);
- to other DMTs in RCTs that addressed all other critical and important outcomes like neoplasms or mortality;
- in any RCTs that addressed quality of life impairment; and

- in any RCTs where people with either relapsing or progressive MS switched to azathioprine after first taking another treatment (Summary of findings 2; Summary of findings 4).

Azathioprine versus placebo or no treatment

Comparisons to placebo or no treatment were not prioritised in this review. However, only the evidence from RCTs to address the benefits and harms of treatment with azathioprine in people with progressive forms of MS used these comparators. We summarise the evidence from RCTs for critical and important priority outcomes here.

Compared to placebo, when treating people with progressive MS, azathioprine:

- may result in a possible decrease in the number of people with disability progression or relapses over three years (very low certainty evidence);
- may result in a possible increase in the number of people with neoplasms over three years (low certainty evidence); and
- may result in a possible decrease in the number of deaths over three years (low certainty evidence).

We found no evidence from RCTs that addressed serious adverse events, short-term adverse events (gastrointestinal disorders) or quality of life in people with progressive forms of MS.

Evidence from NRSIs

We considered evidence from NRSIs in case it could provide information not available from the RCTS, especially for long-term and adverse outcomes and rarer phenotypes of multiple sclerosis. We found that, for the most part, the available evidence from NRSIs does not improve our ability to draw conclusions about the benefits and harms of azathioprine in people with MS. Of the six NRSIs eligible for inclusion according to our objectives and PICO, four could not be included in the synthesis because they were judged to be at critical risk of bias. The remainder mainly provided evidence already available from RCTs, including very uncertain effects on disability, relapse, short-term adverse events and neoplasms, and only in people with RRMS.

The only outcome for which we found evidence in NRSIs that was not available from RCTs was quality of life. We identified evidence from a single, very small, non-randomised study that found individuals with relapsing remitting multiple sclerosis treated with azathioprine versus interferon beta-1b may have higher mental composite and physical composite scores according to the MSQOL-54 questionnaire. However, we graded the evidence as very low certainty.

Overall completeness and applicability of evidence

We included all eligible RCTs and NRSIs of azathioprine for the treatment of multiple sclerosis up to 9 August 2023. We evaluated evidence from 14 studies described in 18 reports. Three ongoing studies met our criteria, and publication of the results may necessitate an update for this review. Please see Characteristics of included studies (and Table 2; Table 3) for the summary of key characteristics of the studies, discussed below.

Population

We included three RCTs and three NRSIs where the population was mixed, in terms of including participants with both relapsing and progressive forms of MS, but data were not presented in subgroups. In order to retain data and address heterogeneity, we applied a threshold of 50% to determine if the study would be considered as one subtype or the other. Such heterogeneity relative to MS phenotype, together with that of MS diagnostic criteria discussed below, limits the applicability of the results in clinical practice.

Considering the populations included in RCTs, the sex/gender balance approximately reflects the natural distribution of MS prevalence with a female-to-male ratio of approximately between 2 and 3 (Alonso 2008), while the population included in NRSIs showed a slightly more even balance between female and male participants, due to the fact that the NRSIs with higher proportion of female participants was small (Milanese 2001) and one study excluded female participants (Swinburn 1973). Despite such small inconsistencies, the sex/gender balance of the included studies does not limit the results.

Overall, the mean age at entry into the study ranged from 27 (Etemadifar 2007) to 42.8 (Putzki 2006) years. Such age ranges are consistent with the typical age at diagnosis (between 20 and 50 years of age, mean 32 years) (Walton 2020).

Three RCTs (Ellison 1989; Goodkin 1991; Milanese 1993) and three NRSIs (Kappos 1990; Amato 1993 ; Confavreux 1996) did not report mean age at study entry or reported mean age at MS onset and mean duration of the condition. The mean age at MS onset amongst participants treated with azathioprine ranged from 27.94 (SD 9.64) (Amato 1993) to 30.7 (± 10.5) (Ellison 1989) years, while amongst participants not treated with azathioprine, it ranged from 29.6 (SD 8.6) (Milanese 1993) to 33.4 (± 9.5) (Ellison 1989) years. Disease duration amongst participants treated with azathioprine ranged from 6.31 (SD 5.93) (Goodkin 1991) to 16.7 (± 10.2) (Ellison 1989) years, while amongst participants not treated with azathioprine, it ranged from 5.37 (SD 7.02) (Amato 1993) to 12.6 (± 5.6) years (Ellison 1989). Ellison 1989 was the only study including 100% of participants with the progressive MS phenotype, and this may explain the longer mean disease duration.

One further consideration regarding the applicability of the evidence related to the population included in the studies is that, given the broad time window covered by our search, MS diagnostic criteria used in the included studies are heterogeneous, spanning from Schumacher 1965 (Ellison 1989; Kappos 1988; Kappos 1990) to McDonald 2001 (Massacesi 2014). Diagnostic criteria incorporating MRI were updated in 2014 (Lublin 2014), and the concept of disease activity changed clinical practice in that DMTs are currently recommended in the early stages of the disease (Rae-Grant 2018). Such a limitation, however, is shared by any condition like MS, where scientific progress leads to periodic updates of diagnostic criteria. In such situations, a compromise has to be adopted between retaining all available data and avoiding heterogeneity in the populations included.

Intervention and previous treatment

No study explicitly addressed the issue of treatment-naive patients versus those switching from a different DMT by means of prespecified subgroup analysis, but in all the RCTs, the exclusion

criteria included previous treatment with immunomodulatory or immunosuppressive drugs. However, the concept of 'previously untreated' was variable: one trial specified that the participants with MS were "previously untreated" (Etemadifar 2007); three trials considered patients who did not receive immunomodulating or immunosuppressive treatments in the preceding year as "previously untreated" (Goodkin 1991; Milanese 1993; Massacesi 2014); one trial excluded people who had taken "cytotoxic agents within the preceding 6 months" (Ellison 1989); one excluded people who had taken "interferon beta therapy, or pulse cyclophosphamide or mitoxantrone in the previous six months", stating that "all patients were naive to interferon beta and none used glatiramer acetate before study entry" (Havrdova 2009); and one excluded patients "on other immunomodulatory drug or hyperbaric oxygen treatment" (British and Dutch 1988).

Further, we only included studies of azathioprine as a generic drug. We did not consider studies of hybrid treatments, such as Jayempi, which contain azathioprine as an active substance. Jayempi is not approved by the FDA, and we are not aware of any RCTs or NRS assessing the effects of this treatment on MS, other than one ongoing study (clinicaltrials.gov/study/NCT03930264), which did not meet our inclusion criteria. However, based on peer reviewer feedback, we may consider such treatments in future updates of this review.

Comparators

We prioritised comparisons of azathioprine with other treatments (Summary of findings 1; Summary of findings 2; Summary of findings 3; Summary of findings 4) over placebo, given the current availability of several DMTs for the treatment of RRMS, as well as of progressive forms of MS. In the included studies, the active comparator was either interferon beta or cyclosporine A. None of the studies included in this review compared azathioprine with more current DMTs, such as S1P receptor modulators (e.g. fingolimod), fumarates (e.g. dimethyl fumarate), CD20 monoclonal antibodies, natalizumab and alemtuzumab, which are widely used today in the treatment of MS in its earlier stages. This limits interpretation of the role of azathioprine in MS treatment.

For the same reason, we prioritised research questions on people with RRMS or progressive MS who need to switch to a different DMT because of lack of benefit or contraindications.

Despite being identified as priority questions, the following were not assessed because the included studies did not report outcomes relevant to them.

- Azathioprine when switching from a different disease modifying therapy compared with other disease-modifying therapies (interferon beta) for relapsing multiple sclerosis
- Azathioprine as a first-choice treatment compared with other disease-modifying therapies (interferon beta) for progressive multiple sclerosis
- Azathioprine when switching from a different disease-modifying therapy compared with other disease modifying therapies (interferon beta) for progressive multiple sclerosis
- Azathioprine when switching from a different disease-modifying therapy compared with placebo for relapsing multiple sclerosis

Outcomes

Of eight RCTs assessing the benefits of azathioprine compared to other disease-modifying treatments or placebo in RRMS and PMS, one RCT on RRMS (Massacesi 2014) and one on PMS (Milanese 1993) reported data about both disability progression and relapses, consistent with our predefined core outcomes. In the remaining RCTs, we could not extract data on the benefits of azathioprine on disability progression, since it was reported as mean differences between groups in the total EDSS score assessed at baseline and at the end of follow-up (British and Dutch 1988; Etemadifar 2007), or by means non-validated tools (Ellison 1989). One small NRSI comparing azathioprine against interferon beta-1b provided very low certainty evidence about relapses and disability progression at one year (Milanese 2001). In 2014, an updated definition of the clinical course and diagnostic criteria of MS was published (Lublin 2014). This update, currently adopted in clinical practice as standard, introduced the definition of 'disease activity' as a broader concept, incorporating both the clinical outcome of recurrence rate and the instrumental outcome of MRI findings. All studies included in this review recruited people with MS before the uptake of this new definition in clinical practice. According to these updated diagnostic criteria, DMTs are currently considered in earlier stages of MS. Therefore, generalisability to current clinical practice of the estimates of efficacy of DMTs from older studies may be limited.

Regarding other prespecified beneficial outcomes, we found very low-certainty evidence from one small NRSI on quality of life (MSQOL-54 scale) at one year compared to interferon beta-1b (Milanese 2001). No evidence was available on cognitive decline. Given these are areas of particular interest for people with MS and clinicians, this represents an important limitation in the evidence needed to assess the benefits and the acceptability of azathioprine.

Treatment discontinuation due to adverse events was chosen as an indirect assessment of azathioprine's acceptability compared to other DMT, but the true compliance rate is not known and was not assessed.

Six out of eight RCTs and no NRSI specified what the authors meant by 'serious adverse events', referring to international classifications of them, and only one RCT fully adopted an international coding system for the definition of adverse events (Table 1), which may create heterogeneity in assessment and limit the applicability of the evidence. None of the NRSIs defined 'serious adverse events'; this was not an outcome in these studies.

Study duration

In terms of comprehensiveness, while we aimed to identify as much relevant information as possible, it should be noted that for most outcomes, follow-up was mainly in the range of two to three years. Being relatively short, such a duration limits the applicability of the results of clinical trials, considering that treatment with DMD in clinical practice is maintained for several years, and the MS course spans decades. We included non-randomised studies looking at long-term outcomes relating to possible harms, though the evidence obtained is mostly very uncertain. This, combined with no reporting or poor reporting, poses a major limitation to determining the overall completeness of the evidence for the benefits and harms of azathioprine.

For the outcome of mortality, in particular, the relatively short duration of trials is an issue, given the rarity of such an outcome in the short term. One RCT involving people with PPMS found a lower mortality rate after three years amongst those treated with AZA than those on placebo (Ellison 1989), while another RCT including mostly people with RRMS found that after three years mortality may be higher amongst people treated with AZA than those on placebo (British and Dutch 1988). The certainty of the evidence is low in both cases. Such results should be interpreted with caution because of the small number of events in each group. In a subgroup of the British and Dutch 1988 study population, 14-year mortality showed an RR estimate in favour of AZA, although with very low certainty.

Study design

We widened the evidence base on harms in the long term by adding the results of NRSIs to the review. Most of the evidence is of poor quality and our certainty in the estimates is very low. One case-control study reported a dose-response relationship between treatment with azathioprine and occurrence of cancer over almost 12 years of follow-up, but this finding is based on few events in a small sample and therefore should be interpreted with caution.

We decided to include NRSIs in our review for three main reasons. First, azathioprine is a treatment whose patent has expired; therefore, new registered clinical trials for azathioprine in the context of the treatment of MS are unlikely. As such, non-randomised controlled studies can be valuable for assessing the benefits and harms of azathioprine in MS. Second, the duration of follow-up of RCTs is usually no longer than three years, and this is a limitation in the assessment of benefits and harms, considering that MS is a chronic condition evolving over decades. Third, one of the main concerns about the long-term use of azathioprine in clinical practice is the potentially increased risk of malignancy associated with cumulative doses of AZA above 600 g, as also underlined in our previous review (Casetta 2007). Such an outcome is relatively rare and, if it were causally related to the exposure to azathioprine treatment, it would require a long-term follow-up in order to be assessed.

Settings

When considering the geographic context in which studies were conducted, the applicability of the evidence appears limited. From the evidence we identified, a location outside of Europe and the USA was specified only in a single study (Iran, Etemadifar 2007). Other than this trial, which was conducted in a low-middle income country (Iran, 94 participants) (Etemadifar 2007), the studies in this review were performed in high-income countries, according to the World Bank Classification: Italy (676 participants), Germany (595 participants), UK (350 participants), USA (126 participants), Czech Republic (118 participants) and The Netherlands (54 participants). This may not entirely reflect the use of azathioprine, which is used in many countries globally, and can be seen as an option in settings with limited resources because it has considerably lower cost than most DMTs, is administered orally and does not need particular storage requirements (Laurson-Doube 2021).

Quality of the evidence

Most of the evidence was of low or very low certainty.

Risk of bias

Our assessment of the risk of bias in the eight included RCTs is summarised in Figure 2 and Figure 3. Four RCTs (50%) were judged to be at high risk of bias due to open label design (participants and treating physicians being aware of which treatment they had been allocated, while outcome assessment was performed by a blinded physician (Etemadifar 2007; Massacesi 2014) (Summary of findings 1) and two studies due to detection bias (lack of blinding in outcome assessment (Goodkin 1991; (Table 7) Ellison 1989 (Table 8)). The former may have occurred since nausea is a frequent short-term adverse event associated with azathioprine, while - amongst active comparators - interferon frequently causes influenza-like illness. In both cases, the occurrence of such symptoms may have suggested to a blinded assessor which treatment group the participants were in.

In several RCTs, some participants were missing from the final analysis after randomisation. However, the risk of attrition bias was always judged as 'low', since: in one study no outcome data were missing (Etemadifar 2007), reasons for missing outcome data were unlikely to be related to true outcome (Kappos 1988; Ellison 1989; Goodkin 1991; Milanese 1993; Havrdova 2009;; Massacesi 2014), missing outcome data were balanced in numbers across intervention groups, with similar reasons for loss across groups (Kappos 1988; Ellison 1989; Goodkin 1991; Milanese 1993; Havrdova 2009;), sensitivity analysis based on imputation of missing data by means of appropriate methods (multiple imputation) was performed (Massacesi 2014), and all randomised participants were reported and analysed in the group to which they were allocated by randomisation, irrespective of non-compliance and co-interventions (Kappos 1988; Ellison 1989; ; Goodkin 1991; Milanese 1993; Etemadifar 2007; Havrdova 2009; ; Massacesi 2014).

Inconsistency

Inconsistency was a reason for downgrading the certainty of the evidence in the core SoF tables (azathioprine compared to interferon as first choice treatment in RRMS) (Summary of findings 1) relative to the short-term outcome nausea/vomiting since the pooled results of two RCTs (Massacesi 2014; Etemadifar 2007) resulted in an I^2 statistic of 85%. Similarly, when considering azathioprine compared to placebo in people with RRMS (Table 7), the certainty in the evidence was downgraded due to an I^2 of 62% when pooling estimates from three RCTs (British and Dutch 1988; Goodkin 1991; Havrdova 2009).

Indirectness

Indirectness was another reason for downgrading the certainty of the evidence in the core SoF tables (azathioprine compared with an active comparator) when considering desirable effects in one study comparing azathioprine with cyclosporine A (Kappos 1988) (Table 6). The population included in the study included both MS phenotypes (relapsing and progressive), and separate outcome data were not provided. Moreover, the timing of disability progression confirmation (ideally six months) was not provided. Amongst studies comparing azathioprine with placebo, indirectness was a reason for downgrading the certainty in the evidence in two studies (Goodkin 1991; Havrdova 2009) (Table 7), adopting a definition of disability progression different from that which was pre-defined in the protocol (confirmation of disability progression assessed at two or three months instead of six). Moreover, in one study (Havrdova 2009), azathioprine was

administered at a dose of 50 mg/day, i.e. half to one third of the dose commonly used in clinical practice (maintenance dose 2.5 to 3 mg per kilogram of body weight per day, 100 to 150 mg per day) (Table 7).

To address heterogeneity while retaining potentially useful data, we adopted a threshold of 50% for the population to be considered as 'progressive' or 'relapsing' (see section Differences between protocol and review) and downgraded the certainty in the evidence whenever such heterogeneity occurred. This type of indirectness was observed in three RCTs (British and Dutch 1988; Kappos 1988; Milanese 1993), two of which (British and Dutch 1988; Kappos 1988) were considered as including participants with RRMS and one (Milanese 1993) was considered as including participants with progressive phenotype of MS. Indirectness due to heterogeneity of populations included was particularly impactful in the comparisons considering the progressive phenotype of MS (Table 8), including about half participants with RRMS and half with progressive forms of MS, without providing separate subgroup data.

Notably, we have been conservative in our certainty judgements by not downgrading for indirectness due to mixed populations when assessing undesirable effects, since there is no reason to think that the adverse events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. This choice was taken after discussion with an international multi-stakeholder guideline development panel that provided input for the definition and prioritisation of outcomes.

Our decisions while assessing certainty on indirectness were made considering that the certainty in the effect on outcomes measuring benefits may be lowered by heterogeneity related to different MS phenotypes, given that RRMS and PMS have different courses and prognosis (e.g. considering relapses as a measure of effect, amongst people with PMS - characterised by a rapidly progressing, chronic disability worsening, independent of relapse - relapses should be assessed separately, otherwise an overall estimate of effect on a mix of people with RMMS and PMS would be poorly generalisable). By contrast, if a drug causes an adverse event due to its mechanism of action, it is likely that it will appear regardless of the phenotype of MS. Therefore, when assessing the certainty in the evidence relative to undesirable effects, we did not consider different phenotypes mixed in the population as a determinant for indirectness.

Imprecision

Imprecision, further reducing certainty of the evidence, was mainly due to wide 95% confidence intervals (often including appreciable benefit as well as appreciable harm), small sample size (and therefore insufficient power to detect the observed difference) and few events (Summary of findings 1; Table 6; Table 7; Table 8).

Whenever wide confidence intervals include the possibility of little or no effect, or of a result favouring the comparator as a possible interpretation of desirable and undesirable effect estimates, our certainty in the point estimate is lowered due to imprecision and therefore these results should be interpreted with caution.

We included six non-randomised studies, all of which were assessed as at least at serious risk of bias overall using the ROBINS-I tool (Figure 4). The studies were variously downgraded in terms of the certainty of evidence because of imprecision, mainly due to small sample size, few events and CIs including appreciable benefit and harm, bias in measurement of outcomes, lack of adjustment for potential confounders and selection bias.

Potential biases in the review process

To limit the risk of publication bias, we searched several principal databases and trial registries to identify published, ongoing and unpublished completed studies, and we applied no restrictions on time or language to the searches. Our search strategies used a range of terms and controlled vocabulary relevant to our prespecified PICO elements (Appendix 1), and we identified a number of further RCTs compared to a previous Cochrane review with comparable PICO (Casetta 2007). In future updates of this review, we may include the following additional search terms, as suggested by one reviewer, to ensure we do not miss any relevant studies due to variations in spelling: encephalo-myelitis (with hyphen) or 'PwMS'.

The current search also identified a number of what appear to be observational reports in local hospital journals that are defunct or otherwise do not provide means to access them. Unfortunately, given the lack of accessible information to assess eligibility, we have 18 reports that are awaiting classification (see Characteristics of studies awaiting classification). We cannot exclude the possibility that these reports hold relevant information, and it is possible that we have not identified other sources of unpublished observational eligible studies. We also identified three ongoing studies, which should be considered in any future updates of this review.

We retrieved fewer than 10 RCTs; therefore, we were not able to exclude the possibility of reporting bias by means of contour-enhanced funnel plots. We compared published results against study protocols to assess for selective reporting. In six out of eight RCTs, a published protocol was unavailable, therefore the risk of reporting bias remains unclear (Characteristics of included studies; Figure 2)

Agreements and disagreements with other studies or reviews

A previously published Cochrane review comparing placebo-controlled RCTs of azathioprine to placebo (Casetta 2007), concluded that azathioprine may be a fair alternative to interferon beta, with a favourable benefit-to-risk ratio. The authors highlighted a need for direct comparisons of azathioprine versus other DMTs, which the current review was able to evaluate, in part, based on newer RCTs. In our review, we partly confirmed this conclusion by finding that, compared to either interferon beta-1b or cyclosporine A, azathioprine for RRMS may have little or no effect on the number of people with disability progression over two years (very low-certainty evidence), but may reduce the numbers of people with relapses significantly more than interferon beta-1b, although our certainty in the evidence is low. The Casetta 2007 review included Ghezzi 1989, which we have placed in Studies awaiting classification as we were unable to obtain a version to evaluate it under the current methods. Unlike the current review, the Casetta 2007 review excluded studies where azathioprine was administered in combination with other treatments and this led to the exclusion of one placebo-controlled study that we included in our review (Havrdova 2009), though this does not appear to have led to substantive differences between the reviews.

Pairwise results for Tramacere 2015 are largely consistent with our findings: low to very low quality evidence indicating azathioprine may improve relapses relative to placebo and interferon over 12 to 24 months; very low certainty evidence that azathioprine may have slight effects on disability progression over 24 months versus placebo and interferon beta; and very low certainty evidence that azathioprine may increase discontinuations versus placebo and interferon over 24 months. Tramacere 2015 conducted a network meta-analysis (which was not performed in the current review), and they did not find significant differences in effects between other DMTs and azathioprine.

A previous network meta-analysis on immunosuppressants and immunomodulators based on placebo as common comparator found that, when pooling the results of studies including people with all types of MS, azathioprine is more effective than other DMTs in reducing the number of people with relapses over 12 and 24 months of treatment (Filippini 2013). In our review, the frequency of relapse was the only 'benefits' outcome that showed an estimate in favour of azathioprine, in line with Filippini 2013.

Filippini 2017 performed a network meta-analysis of treatment with DMTs for people with a first clinical attack suggestive of multiple sclerosis. Azathioprine was included amongst the eligible interventions, but they did not find enough evidence relating to azathioprine to include it in analyses for this distinct population group, and therefore drew no conclusion regarding the benefits and harms of azathioprine.

A recent Cochrane review compared adverse effects of immunotherapies for people with MS or clinically isolated syndrome (Tramacere 2023), and the authors of that review ranked these treatments according to their relative risks of adverse effects through network meta-analysis. Consistent with the results of the present review, the authors were very unsure about unwanted effects causing people to stop taking azathioprine because the evidence regarding dropouts was of very poor quality.

Similarly to our findings, all previously mentioned Cochrane reviews have noted the lack of insufficient high-quality data upon which to make definitive conclusions regarding the benefit-risk balance of azathioprine.

We widened the evidence base relative to harms in the long term by adding the results of NRSIs to the review. Most of the evidence is of poor quality and our certainty in the estimates is very low. One case-control study, Confavreux 1996, reported a dose-response relationship between treatment with azathioprine and occurrence of cancer over almost 12 years of follow-up, but this finding is based on few events in a small sample (92 participants) and therefore should be interpreted with caution.

AUTHORS' CONCLUSIONS

Implications for practice

Azathioprine has been used or proposed as an alternative to approved on-label disease-modifying treatments (DMTs) for multiple sclerosis (MS) when access to the latter is restricted, especially in low-resource settings (Laurson-Doube 2021; Rae-Grant 2018; Yamout 2019). The limited available evidence identified in this review suggests that, compared to interferon beta, treatment with azathioprine may result in a possible decrease in relapse frequency (low-certainty evidence), and an increase in serious adverse events (low-certainty evidence) in people with relapsing remitting MS. It also suggested that there may be a possible decrease in the progression of disability and possible increase in short-term gastrointestinal adverse events, but the evidence for these was very uncertain (very low certainty evidence). None of the studies included in this comparison assessed our other key outcomes of interest: quality of life impairment (mental score), long-term adverse events (neoplasms) and mortality.

It was not possible to come to conclusions about the relative benefits and harms of azathioprine in people with progressive MS compared to other DMTs based on the evidence in this review, which included only comparisons to placebo or no treatment. Further, overall, the evidence from non-randomised studies of interventions did not provide any additional evidence beyond that available from randomised controlled trials.

These conclusions should be interpreted cautiously because the certainty of the evidence is low and very low, and the effect estimates may be biased. Moreover, the applicability of reported estimates is poor, given the methodological limitations and the short follow-up periods of most of the trials.

Implications for research

We identified a high risk of bias in most of the trials included in this review; in particular, detection bias in the randomised controlled trials (RCTs). Moreover, imprecision is a concern due to small sample sizes and the small number of events. Well designed and executed studies are needed that involve a large number of participants and ensure outcome assessors are blinded to participants' treatment group.

More direct evidence that is pertinent to real-world experience of treating people with MS with azathioprine is needed to draw firmer conclusions about the benefits and harms of azathioprine. This includes the need - which may be partially addressed by Ongoing studies - for evidence from head-to-head comparisons of azathioprine versus a greater range of the active comparators that are in current use for the treatment of MS. Although we considered any comparison of azathioprine versus any other DMT possibly used for treating MS, regardless of the drug class or mechanism of action, data on azathioprine versus active comparators were available only for interferon and cyclosporine A. Newer DMTs, such as S1P receptor modulators (e.g. fingolimod), fumarates (e.g. dimethyl fumarate), CD20 monoclonal antibodies, natalizumab and alemtuzumab, are now widely used in clinical practice to treat MS. However, azathioprine has not been compared to any of them in clinical trials. This limits interpretation of the role of azathioprine in MS treatment, even in low-resource areas. Furthermore, studies considering a diversity of settings, including those with limited resources, would improve the directness of evidence.

People with MS may change treatment multiple times, but we found no evidence that investigated benefits and harms when switching to azathioprine. Adverse effects, especially over the long-term, are an important consideration, but our ability to draw conclusions about this issue is limited as the available studies followed participants for only two or three years. All the abovementioned limitations are especially the case for people with progressive forms of MS with azathioprine, for whom we found

no evidence comparing azathioprine to any active comparators whatsoever and no RCT evidence for serious adverse events.

Outcomes that are important to patients, like quality of life and cognitive status, were largely absent in the evidence. We found some evidence from non-randomised studies, but this was of very low certainty and only available for relapsing forms of MS. This gap in the evidence represents an important limitation in any effort to assess the comparative effectiveness and acceptability of azathioprine.

ACKNOWLEDGEMENTS

We thank Chiara Bassi (Azienda USL – IRCCS di Reggio Emilia) and Maria Domenica Camerlingo in their capacity as Information Specialists for the Cochrane Multiple Sclerosis and Rare Diseases of the CNS Group. They contributed to developing the search strategy for this review and to retrieving full-text articles that were not otherwise available. We also thank Robin Featherstone, Information Specialist, Cochrane Central Executive Team, who developed the search strategy for this review.

The publication of this article was supported by the 'Ricerca Corrente' funding from the Italian Ministry of Health.

Editorial and peer reviewer contributions

Cochrane Multiple Sclerosis and Rare Diseases of the CNS Group supported the authors in the development of this intervention review.

The following people conducted the editorial process for this article.

- Sign-off Editor (final editorial decision): Robert Boyle, Imperial College London
- Managing Editor (selected peer reviewers, provided editorial guidance to authors, edited the article): Joanne Duffield, Cochrane Central Editorial Service
- Editorial Assistant (conducted editorial policy checks, collated peer-reviewer comments and supported editorial team): Leticia Rodrigues, Cochrane Central Editorial Service;
- Copy Editor (copy editing and production): Cochrane Central Production Service
- Peer reviewers (provided comments and recommended an editorial decision): Jennifer Hilgart, Cochrane (methods); Ina Monsef, Cochrane Haematology, Department I of Internal Medicine, Center for Integrated Oncology, Aachen, Bonn, Cologne, Duesseldorf, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany (search); Marisa McGinley, Cleveland Clinic Mellen Center, Ohio, USA (clinical); Loredana La Mantia, Fondazione Don Carlo Gnocchi, Milano, Italy (clinical); Clarinda Cerejo, Patient and Patient Advocate, Mumbai, Maharashtra, India (consumer).

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Amato 1993

Study characteristics	
Methods	**Design:** retrospective, controlled cohort study, 2 arms **Blinding:** NA **N of centres:** 1 centre in Italy **Inclusion criteria** • Referral to the Department of Neurology of the University of Florence from 1 January 1978 to 31 December 1990 • At least 3 months of treatment with azathioprine with daily dose of about 2 mg/kg body weight **Exclusion criteria:** prior history of neoplasm **Duration:** mean 4.16 (SD 2.14) years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included:** 454 **Phenotype:** RRMS N = 329 (73%); chronic progressive N = 70 (15%); remitting progressive N = 55 (12%) **Diagnostic criteria:** not stated **Sex/gender:** N = 301 female participants (66% of included participants), N = 153 male participants **Mean age at study entry:** reported only age at disease onset **Disease duration:** reported only duration of treatment and first evaluation in study centre **EDSS at entry:** not stated
Interventions	**Intervention:** N = 207 (46% of participants) treated with azathioprine (at least 3 months of treatment with azathioprine, mean daily dose 110.51 mg (SD 19.84, range 100 to 150)). **Comparator:** N = 247 (54% of participants) not treated with azathioprine
Outcomes	Diagnosis of malignancy confirmed by histology in azathioprine cohort and in non-azathioprine cohort; age- and sex-adjusted neoplasm frequency rate; mean treatment period for participants with malignancy outcome; adverse events (clinical and instrumental) in azathioprine group

Amato 1993 *(Continued)*

Notes	**Funding:** not stated **Conflicts of interest:** not stated, funding from private philanthropic organisations

British and Dutch 1988

Study characteristics	
Methods	**Design:** parallel RCT, 2 arms; superiority **Blinding:** double **N of centres:** 20 hospitals in UK and Holland **Inclusion criteria** • Clinically definite MS (at least two episodes and two clinical lesions or two episodes and one subclinical lesion) • Laboratory confirmed MS (at least two anatomically separate episodes, one clinical lesion and oligoclonal bands or increased IgG in the cerebrospinal fluid) • Currently progressive MS (two necessarily separate lesions, of which one might be subclinical, oligoclonal bands or increased IgG in the CSF, and progression for at least 6 months • Patients with RRMS in remittent or stationary phase for 1 month or longer at entry who have had at least one relapse in the previous year • Ambulant (EDSS 6 or less) • Age 15 to 50 years • Not on other immunomodulatory drug or hyperbaric oxygen treatment **Exclusion criteria** • Current treatment with other immunomodulatory drugs or hyperbaric oxygen • Concomitant systemic disease and mental deficit **Duration:** 3 years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included** • N randomised: 354 (N = 174 azathioprine, N = 180 placebo) • N = 332 followed participants at 3 years **Phenotypes:** RRMS N = 236 (67% of randomised participants); SPMS N = 67 (18% of randomised participants); PPMS N = 51 (15% of randomised participants) **Diagnostic criteria:** Poser 1983 **Sex/gender:** N = 207 female participants (58% of randomized participants), N = 147 male participants **Mean age at study entry:** 39 (SD 8.6) years (azathioprine); 38 (SD 8.3) years (placebo) **Disease duration:** mean 9 years **DSS at entry:** mean 3.69 (SD 1.05) (azathioprine); mean 3.66 (SD 1.62) (placebo) 300 UK participants followed up in the Taylor 2004 paper for this study (see study references) Subset of Dutch participants reported at same time point in the Minderhoud 1988 paper for this study (see study references)

British and Dutch 1988 *(Continued)*

Interventions	**Intervention:** N = 174 (N = 161 followed at 3 years), azathioprine 2.5 mg/kg/day (to the nearest 25 mg) **Comparator:** N = 180 (N = 171 followed at 3 years), placebo
Outcomes	Mean change in EDSS score, Kurtzke Functional Scales and Ambulation Index at 1, 2 and 3 years Mean number of relapses per participant per year at 1, 2 and 3 years Adverse events (clinical and instrumental) at 3 years Cancer rates and mortality at 15 years follow-up for the 300 UK participants randomised in the study are reported in Taylor 2004
Notes	**Funding:** by the Medical Research Council. Wellcome Research laboratories supplied azathioprine and placebo tablets **Conflicts of interest:** not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided on the method used to generate the allocation sequence
Allocation concealment (selection bias)	Unclear risk	No information provided on the method used to conceal the allocation sequence
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Quote: "The patients were also seen by a non-masked doctor who had access to laboratory results and prescribed the trial medication."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Disability status assessments performed by "masked assessor". Quote: "To preserve masking where a patient on active treatment had to have their treatment changed because of abnormal laboratory values, another patient on placebo at that centre was asked to make a similar change in their treatment."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "More than 90% of patients attended for follow-up at 12, 24, and 36 months after trial entry." Quote: "The main analyses were conducted on all the patients with follow-up data."
Selective reporting (reporting bias)	Low risk	Prespecifed outcomes and analyses available
Other bias	Low risk	Study appeared free of other sources of bias

Confavreux 1996

Study characteristics

Methods	**Design:** case-control study **Blinding:** NA

Confavreux 1996 *(Continued)*

	N of centres: 1 centre in France **Inclusion criteria** • MS patients with and without cancer collected through the Lyon Multiple Sclerosis Database, implemented in 1990, including all patients with a diagnosis of MS admitted at least once at the Clinique de Neurologie, Hopital Neurologique, Lyon, since 1957 • Diagnosis of MS • Cases: pathologically confirmed diagnosis of malignant neoplasm of solid tissue (codes 140 through 195 in the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CMI) or of malignant neoplasm of lymphatic and hematopoietic tissue (ICD-9-CM codes 200 through 208), known to have developed cancer after the clinical onset of MS and before 31 December 1991 • Controls: matching variables: sex, date of birth (± 5 years) and date of MS onset (± 5 years) **Exclusion criteria:** insufficient confidence in the MS diagnosis **Duration:** variable given study design, 0 to ≥ 10 years treatment/follow-up **Intention-to-treat analysis:** NA
Participants	**N included:** 92 (case-control ratio 1:3) **Phenotypes:** information provided only on the "Initial course"; RRMS 74% of cases and 83% of controls, the remainder being PMS **Diagnostic criteria:** Poser 1983 **Sex/gender:** states only "By design, the two groups were similar in terms of gender (57% female) and duration of follow-up taken into account" **Mean age at study entry:** not stated, provided only age at MS onset **Disease duration:** states "The average time interval from clinical onset of MS to cancer diagnosis was 13.8 ± 8.1 years (median, 14 years; range, 0.1 to 29.1)" **EDSS at entry:** not stated
Interventions	**Exposure:** N = 23 participants; treatment with azathioprine within the time interval between MS onset and the diagnosis of cancer for cases for at least one month and at a cumulative dose of least 5 g **Controls:** N = 69 participants; people with MS included in the database who were free of any malignancy at the time the matched case was diagnosed with cancer. Azathioprine therapy taken after this time window was not taken into account.
Outcomes	Risk (odds ratio) of cancer related to azathioprine exposure, compared to no azathioprine exposure; total duration of AZA intake and cumulative dose were considered as variables in a logistic regression to assess dose-response relationship
Notes	**Funding source:** Hospices Civils de Lyon and grants from the Commission of the European Communities DG XI1 (contract no. BMHl-CT93-1529), the Ligue Francaise Contre la Sclerose En Plaques, and the Nouvelle Association Française des Scleroses en Plaques **Conflicts of interest:** not stated

Ellison 1989

Study characteristics	
Methods	**Design:** parallel RCT, 3 arms; superiority

Ellison 1989 *(Continued)*

	Blinding: double **N of centres:** 1 centre, USA **Inclusion criteria** • Age 18 years or older • Living within 150 miles from the clinic and intend to stay in the area for the next 3 years • Transportation available • Eat independently • Transfer to and from wheelchair with no assistance • Mentally competent and able to provide consent **Exclusion criteria** • Pregnancy or pregnancy planned within next 3 years • Men wishing to father offspring within next 3 years • Infections not under treatment • Pressure ulcers • Active coccidiomycosis • Past or present neoplastic diseases • Diseases that compromise neurological assessment (deforming arthritis, major amputations, psychoses) • Cytotoxic therapy within the preceding 6 months • Steroids within the preceding 3 months • Relapses within the preceding 3 months **Duration:** 3 years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included** • N randomised: 67 (N = 33 azathioprine, N = 34 placebo); N = 2 randomised to the azathioprine group dropped out before starting tratment • N = 54 participants followed at three years (N = 26 azathioprine, N = 28 placebo) **Phenotype:** PMS N = 67 (100% of randomised participants) **Diagnostic criteria:** Poser 1983, Schumacher 1965 **Sex/gender:** N = 31 female participants (46% of randomised participants), N = 34 male participants. Sex/gender not provided for the 2 participants randomised in the azathioprine group who dropped out before starting tratment **Mean age at study entry:** not reported **Disease duration:** mean 16.7 (SD 10.2) years (azathioprine); mean 12.6 (SD 5.6) years (placebo) **DSS at entry:** mean 5.6 (SD 1.2) (azathioprine); mean 5.5 (SD 1.0) (placebo)
Interventions	**Intervention:** N = 33 (N = 26 followed at three years), azathioprine started at 2.2 mg/kg/day (to the nearest 25 mg) up to above 4.4 mg/kg/day until the white blood cell count was maintained between 3000 to 4000 or adverse events were encountered. Placebo intravenous preparation added **Comparator:** N = 34 (N = 28 followed at three years), placebo
Outcomes	Illness Severity Score (ISS); Standard Neurologic Examination Score (SNE) (progression rates); mean difference DSS, ISS and SNE scores at 3 years (ending minus baseline); number of participants who

Ellison 1989 *(Continued)*

	worsened defined as a change in DSS over 3 years; number of relapses for participant at 1, 2 and 3 years; number of participants relapsed at 3 years; adverse events (clinical and instrumental)
Notes	**Funding source:** supported by USPHS grants and grants from the Gustafson Estate and Joe Ghaen Barbecue Fund, Department of Neurology, UCLA **Conflicts of interest:** not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation process using blocks of 4 successive patients
Allocation concealment (selection bias)	Low risk	Quote: "The statistician told the examining neurologists that the tratments would be allocated by a randomization process to block of 4 successive patients, but the assignment rules were not revealed. A master list was computed in which treatments were assigned according to patient sequence number. Patient sequence was the order of presenting the initial prescription to the pharmacy"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "Observer and monitor neurologist, study nurse, clinical coordinator NP technician and patients were masked to treatment assigned". Quote: "The apparent detection of treatment groups by the monitoring neurologists, clinic coordinator and nurse probably occurred because they had access to laboratory reports"
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "The apparent detection of treatment groups by the monitoring neurologists, clinic coordinator and nurse probably occurred because they had access to laboratory reports" A high percentage of the clinicians who monitored the participants and assessed the outcomes correctly guessed to which arm the participants had been randomised. 61% of participants randomised to placebo correctly guessed their assignment.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Participants followed for 3 years were 26/33 and 28/34 in the AZA and placebo arms, respectively. Causes of loss to follow-up were similar in the two groups.
Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Etemadifar 2007

Study characteristics

Methods	**Design:** parallel RCT, 2 arms; superiority **Blinding** single (concealed assessment) **N of centres:** 1 centre, Iran **Inclusion criteria**

Etemadifar 2007 *(Continued)*

- Age 13 to 50 years
- ≥ 2 relapses within the 2-year period of treatment initiation
- EDSS score ≤ 5 with at least 2 years of a natural disease course
- No previous treatment with DMTs

Exclusion criteria

- Evidence of neurological, psychiatric, cardiac, endocrinological, haematological, hepatic, renal, pulmonary disease, active malignancy
- Autoimmune diseases, or other chronic diseases
- History of uncontrolled seizure or suicidal ideation or an episode of severe depression within 3 months before enrolment
- Lactation and pregnancy as determined by history, physical examination and screening blood tests

Duration: 1 year treatment/follow-up

Intention-to-treat analysis: performed

Participants	**N included** • N randomised: 94 (N = 47 azathioprine, N = 47 interferon-beta products) • N = 94 participants followed at 1 year **Phenotype:** RRMS N = 94 (100% of randomised participants) **Diagnostic criteria:** Poser 1983 **Sex/gender:** N = 74 female participants (79% of randomised participants), N = 20 male participants **Mean age at study entry:** 27.1 (SD 8.8) years (azathioprine group), 28.3 (SD 6.8) years (interferon-beta products group) **Disease duration:** not reported **EDSS at study entry:** mean 1.4 (SD 0.3) (azathioprine group); 1.6 (SD 0.6) (interferon-beta products group)
Interventions	**Intervention:** N = 47 (47 followed at 1 year), azathioprine starting dose 25 mg orally, once a day, for the first week, then increase by 25 mg weekly until reaches 3 mg/kg per day in divided dosage, as tolerated, according to symptomatic side effects or until haematological or chemical toxicity (transaminitis or neutropenia) occurred. Dose reduction by 25% if WBC fell to 3000 to 3500 or the SGOT or SGPT reached 2 to 3 times the upper limit of normal. Likewise, dose reduction by 50% if WBC fell to 2500 to 3000 or SGOT or SGPT increased 3- to 4-fold **Comparator:** N = 47 (47 followed at one year), interferon-beta products regimen: (N = 15 Betaferon 250 µg sc every other day injection, N = 19 Avonex 30 µg im once weekly, and N = 13 Rebif 44 µg sc 3 times weekly)
Outcomes	EDSS score before treatment and after 12 months within-arm and between-arms (at 3, 6 and 12 months); mean number of relapses; adverse events (clinical and instrumental)
Notes	**Funding source:** not stated **Conflicts of interests:** not stated

Risk of bias

Bias	Authors' judgement	Support for judgement

Etemadifar 2007 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Quote: "Patients were randomized according to a preexisting list produced by a computer program that differed from a random number generator only in that it assigned equal numbers of patients into each treatment group."
Allocation concealment (selection bias)	Unclear risk	No information provided on how assignment was blinded
Blinding of participants and personnel (performance bias) All outcomes	High risk	Single-blind (participants were aware of treatment)
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Assessment was concealed, but it is possible that knowledge of treatment allocation may have biased participants' reporting of subjective outcomes, such as short-term adverse events (headache, nausea).
Incomplete outcome data (attrition bias) All outcomes	Low risk	3/47 in AZA group and 3/47 in IFN group stopped the assigned treatment, all due to adverse events, but were followed up for 12 months
Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Goodkin 1991

Study characteristics	
Methods	**Design:** parallel RCT, 2 arms; superiority **Blinding:** double **N of centres:** 1 centre, USA **Inclusion criteria** • No exacerbation during the 1-month period prior to study entry • No chronic progressive disease over 6 months without associated exacerbation • Age 18 to 65 years • Entry EDSS score 2.0 to 6.5, inclusive, and an Ambulation Index score of 1.0 to 6.0, inclusive • No corticosteroids during the 1 month before study entry, no immunosuppressant medication for 1 year before entry, or total lymphoid irradiation at any time **Exclusion criteria** • Pregnancy • Unwillingness to practice birth control during the study period • Systemic illness or medical condition precluding safe administration of azathioprine • Incapability of understanding the requirements of the study or of signing informed consent **Duration:** 2 years treatment/follow-up **Intention-to-treat analysis:** not performed
Participants	**N included**

Goodkin 1991 *(Continued)*

- N randomised: 59 (N = 30 azathioprine, N = 29 placebo)
- N = 52 participants followed at two years

Phenotype: RRMS N = 59 (100% of randomised participants)

Diagnostic criteria: Poser 1983

Sex/gender: 36 female participants (61% of randomised participants), 18 male participants

Mean age at study entry: not reported

Disease duration: mean 6.31 (SD 5.93) years (azathioprine group), mean 6.24 (SD 8.34) years (placebo group)

EDSS at entry: mean 3.2 (SD 1.2) (azathioprine group); mean 3.7 (SD 1.6) (placebo group)

Interventions	**Intervention:** N = 30 (N = 27 followed at two years), azathioprine target dose of 3 mg/kg reached with increases of 25 mg/day no more frequently than once per month. Weekly monitoring of CBC, SGOT and SGPT for 4 weeks and monthly thereafter. Leukocyte (WBC) count maintained between 3500 and 4000 per µ1. Dose reduction of 25% when WBC 3000 to 3500 per µ1 or values 2 to 3 times the upper limit of normal for SGOT or SGPT, and 50% for WBC 2500 to 3000 per µ1, or values 3 to 4 X ULN for SGOT or SGPT **Comparator:** N = 29 (N = 25 followed at two years), placebo
Outcomes	N of participants experiencing on-trial exacerbations at 2 years; progression of disability, as measured by mean change in the EDSS at 2 years; time to first relapse, percentage of groups sustaining exacerbation at 2 years, time to deterioration in EDSS sustained for > 2 months, change in mean Ambulation Index (AI) score at 2 years, time to deterioration in AI score sustained for > 2 months, time to deterioration of at least 20% in baseline nine-hole-peg test (9HPT) or box-and-block test (BBT) sustained for > 2 months, percentages of groups experiencing at least 20% deterioration in baseline 9HPT or BBT sustained for > 2 months over 2 years, participant's subjective assessment of treatment outcome at 2 years, examining physician's assessment of treatment outcome after review of medical records obtained during the 2 years on trial; adverse events (clinical and instrumental) over 2 years
Notes	**Funding source:** supported in part by the National Multiple Sclerosis Society (RG-1762-A-1); medications supplied by the Burroughs Wellcome Company **Conflicts of interest:** not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed by means of random number table prepared by a statistician.
Allocation concealment (selection bias)	Unclear risk	No details provided on allocation concelament
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding not effective on participants: 44% correctly guessed what treatment they had been assigned to. Treating neurologist unmasked
Blinding of outcome assessment (detection bias) All outcomes	High risk	Blinding not effective on participants, and unblinding of treating physicians may have affected blinding of assessors
Incomplete outcome data (attrition bias)	Low risk	Proportion of participants lost to follow-up and reasons for loss were similar in the two arms of the trial.

Goodkin 1991 *(Continued)*
All outcomes

Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Havrdova 2009

Study characteristics	
Methods	**Design:** parallel RCT, 3 arms; superiority **Blinding:** double **N of centres:** not stated, Czech Republic **Inclusion criteria** • Age 18 to 55 years • EDSS score ≤ 3.5 at entry • Active disease (2 relapses in the last 12 months or 3 relapses in the last 24 months) • Women of childbearing potential willing to use effective contraception • Immunomodulating therapy to be terminated at least 4 weeks before study entry **Exclusion criteria** • Interferon-beta therapy in the previous 12 months or glatiramer acetate before study entry • Immunosuppressive treatment with either pulse cyclophosphamide or mitoxantrone in the previous 6 months • Pregnancy or using ineffective contraception methods • Active major organ disease, especially hepatic and endocrine disorders **Duration:** 2 years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included** • N randomised: 118 (N = 58 azathioprine and interferon beta-1a, N = 60 interferon beta-1a and placebo) • N = 116 participants followed at two years **Phenotype:** RRMS N = 118 (100% of randomised participants) **Diagnostic criteria:** Poser 1983 **Sex/gender:** N = 98 female participants (83% of randomised participants), N = 20 male participants **Mean age:** 30.9 (SD 7.8) years (azathioprine and interferon beta-1a), 28.9 (SD 7.1) years (interferon beta-1a and placebo) **Disease duration:** mean 5.5 (SD 5.2) years **EDSS:** mean 1.9 (SD 1.1) (azathioprine and interferon beta-1a); mean 1.8 (SD 0.9) (interferon beta-1a and placebo)
Interventions	**Intervention:** N = 58 (N = 58 followed at 2 years), im interferon beta-1a 30 µg once weekly and azathioprine 50 mg once daily, and placebo every other day

Havrdova 2009 *(Continued)*

	Comparator: N = 60 (58 followed at 2 years), im interferon beta-1a 30 µg once weekly and placebo
Outcomes	Annualised relapse rate at 2 years (relapses defined as the appearance or reappearance of one or more symptoms attributable to MS, accompanied by objective deterioration on neurologic examination, lasting at least 24 h, in the absence of fever, and preceded by neurologic stability for at least 30 days); time to first relapse; proportion of patients with clinical relapses at 2 years; time to sustained progression, progression of disability being measured by an increase in EDSS score (increase of ≥ 1.0 point from a baseline score > 0 or an increase of ≥ 1.5 points from a baseline score of 0) sustained for 12 weeks at 2 years; accumulation of T2-lesion volume and brain atrophy as measured by percentage brain volume change at 2 years; adverse events (clinical and instrumental) at 2 years
Notes	**Funding source:** MRI acquisition and analysis, first draft of the paper, technical and editorial support during the preparation for submission supported by Biogen Idec. Support from the Czech Ministry of Education (research program MSM 0021620849) **Conflicts of interest:** funding includes commercial entities with potential interests who were involved in the preparation of the article.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Stratified randomisation by means of centralised randomisation schedule
Allocation concealment (selection bias)	Unclear risk	No information provided on the method used to conceal the allocation sequence
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients were randomized in a 1:1:1 ratio to one of three treatment groups: IM interferon beta-1a 30 µg onceweekly plus placebo AZA orally (p.o.) once daily, plus placebo corticosteroid p.o. every other day; IM interferon beta-1a 30 µg once weekly plus AZA 50 mg p.o. once daily, plus placebo corticosteroid p.o. everyother day; or IM interferon beta-1a 30 µg once weekly plus AZA 50 mg p.o. once daily, plus prednisone 10 mgp.o. every other day." Quote: "All study personnel were blinded to treatment assignment, except in the event of a medical emergency or as required by regulatory authorities."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Separate personnel were designated to conduct efficacy assessments and to treat patients to protect against perceived unblinding of study drug assignment."
Incomplete outcome data (attrition bias) All outcomes	Low risk	None of the 58 participants in the AZA + interferon group was lost to follow-up. 2/58 were lost to follow-up for non-compliance in the placebo + interferon group. Unlikely to bias final results
Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Kappos 1988

Study characteristics

Methods	**Design:** parallel RCT, 2 arms; superiority

Kappos 1988 *(Continued)*

Blinding: double, participants and physicians (only the physician responsible for the laboratory monitoring and the study nurse in each centre were informed of treatment group)

N of centres: 3 (Germany, Hannover and Wuerzburg)

Inclusion criteria

- Elevated autochthonous IgG production in the central nervous system and/or oligoclonal bands in the cerebrospinal fluid
- Active disease during the past 2 years (either well-documented occurrence of more than one relapse per year or deterioration of 1 or more points in EDSS during the previous year)
- Age 18 to 50 years
- No other immunosuppressive treatment during the past 2 years (patients treated with azathioprine admitted without washout)
- EDSS score 0 to 6.5
- Interval of at least 10 weeks between the start of the last relapse and inclusion in the study
- No medical illnesses or psychic alterations judged incompatible with safe administration of the treatment regimens
- Good compliance

Exclusion criteria: not stated

Duration: 2 years treatment/follow-up

Intention-to-treat analysis: not performed

Participants	**N included** • N randomised: 194 (N = 96 azathioprine, N = 98 cyclosporine A) • N = 167 participants followed at 2 years; N = 27 (28%) of those randomised in the azathioprine group and N = 26 (27%) of those randomised in the cyclosporine A group had been previously treated with azathioprine **Phenotypes:** RRMS N = 123 (63% of randomised participants), RPMS N = 46 (24% of randomised participants), chronic progressive MS N = 25 (13% of randomised participants) **Diagnostic criteria:** Schumacher 1965 **Sex/gender:** 126 female participants (65% of randomised participants), 68 male participants **Mean age:** 34.7 (SD 9.0) years (azathioprine), 35.5 (SD 8.4) years (cyclosporine A) **Disease duration:** mean 7.2 (SD 6.9) years (azathioprine group), mean 6.1 (SD 5.1) years (cyclosporine A group) **EDSS:** 0 to 2.5: 44 (46%) azathioprine, 45 (46%) cyclosprine A; 3 to 4.5: 34 (35%) azathioprine, 36 (37%) cyclosporine A; 5 to 6.5: 18 (19%) azathioprine, 17 (17%) cyclosporine A
Interventions	**Intervention:** N = 96 (82 followed at two years), azathioprine tablets 2.5 mg/kg/day and placebo drinking solution. Azathioprine dosage increased stepwise if the mean corpuscular volume of the erythrocytes did not exceed the upper limit of normal after 6 months of treatment. Reduced by 25% if the leukocyte count fell more than 500/mm^3 below the normal limit or if creatinine values were greater than 130 pmoles/l, if liver enzymes exceeded the upper limit of normal, or when other clinical or instrumental severe side effects occurred **Comparator:** N = 98 (85 followed at 2 years), cyclosporine A drinking solution 5 mg/kg/day and placebo capsules. Cyclosporine dosage adjusted to obtain trough whole blood levels between 200 and 1000 ngl ml. Therapeutic range lowered to values between 150 and 750 ngl ml after the first 9 months.
Outcomes	N of participants with disability worsening (measured by means of differences in Neurostatus Score, EDSS score, Ambulation Index, Incapacity Scale at entry and after 2 years; polynomial approximation of Neurostatus score and EDSS score); mean annual relapse rate; N of participants reporting adverse

Kappos 1988 *(Continued)*

	events (clinical and instrumental, up to 32 months); N of participants withdrawn due to adverse events over 2 years
Notes	**Funding source:** supported by funds of Sandoz AG and Herman and Lilly Schilling Foundation **Conflicts of interest:** not stated, but funding includes commercial entities with potential interest

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "stratified randomisation schedule"
Allocation concealment (selection bias)	Unclear risk	No details provided about allocation concealment
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients and all the physicians directly involved in the care and evaluation of the patients were masked for treatment group."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Each neurological evaluation...was performed by the same neurologist who had no access to the previous examinations."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Proportion of participants recorded as dropped out at the end of the 24-month follow-up were similar in the two groups, and the causes for dropping out were balanced.
Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Kappos 1990

Study characteristics

Methods	**Design:** retrospective, matched-pairs controlled study, 2 arms **Blinding:** NA **N of centres:** one centre in Germany **Inclusion criteria** • Well-documented neurological examination at the beginning of the observation period (not during an acute relapse) • Age less than 55 years at the beginning of the observation period • Residence within 150 km of the study centre • Ambulatory at the beginning of the observation period (EDSS score of 6.5 or less) • Documented treatment with azathioprine for ≥ 2 years or no immunosuppressive treatment at all (short-term steroid treatment in acute relapse was not regarded as immunosuppressive in this context) • Selected participants with follow-up available after 10 years: N = 79 people with MS; 41 treated with azathioprine, 38 non treated with immunosuppressors matched by EDSS score, sex, age (± 4 years)

Kappos 1990 *(Continued)*

	and duration of disease. Among these, EDSS score was determined in 28 people with MS (17 treated with azathioprine, 11 non treated with immunosuppressors) that could be re-examined at the end of the 10-year follow-up **Exclusion criteria:** not specified **Duration:** 10 years of treatment/follow-up **Intention-to-treat analysis:** not performed
Participants	**N included** • N = 84 • N = 79 participants followed at 10 years **Phenotypes:** unclear (clinically definite diagnosis of RMS or PMS, proprotions not stated) **Diagnostic criteria:** Schumacher 1965 **Sex/gender:** 49 female participants (62% of participants), 30 male participants **Mean age at study entry:** unclear, provided only for various subgroups **Disease duration:** unclear, provided only for various subgroups **EDSS at entry:** unclear, provided only for various subgroups
Interventions	**Exposure:** N = 41 participants, treatment with azathioprine for at least 2 years during follow-up. Prescribed dosage was 2 to 2.5 mg/kg body weight **Control:** N = 38 participants with MS not treated with any immunosuppressive agent
Outcomes	EDSS score, Neurostatus and Ambulation Index at entry and at the end of the 10-year follow-up, mortality
Notes	**Funding source:** Lilly Schilling Foundation **Conflict of interest:** not stated, funding from private philanthropic foundation **Comment:** the diagnostic ascertainment according to the predefined criteria and the health status of patients with MS not checked for more than 2 years was assessed by sending a questionnaire to the patient's physician and, if necessary, by contacting the patient or her/his relatives by phone. Re-examination consisted in assessment of neurostatus score, EDSS and Ambulation Index. Self-reported compliance to treatment with azathioprine was considered as a variable for analysis.

Massacesi 2014

Study characteristics	
Methods	**Design:** parallel RCT, 2 arms; non-inferiority **Blinding:** single-blind (concealed assessment) **N of centres:** 30 centres in Italy **Inclusion criteria** • Age 18 to 55 years • At least 2 clinical relapses in the preceding 2 years • Baseline EDSS 1.0 to 5.5

Massacesi 2014 *(Continued)*

	• Effective female contraception and a signed informed consent **Exclusion criteria** • Clinical relapses or steroid therapy 30 days prior to study entry • Immunomodulatory/immunosuppressive treatments in the previous year • Concomitant diseases precluding IFN or azathioprine treatment • Pregnancy or breastfeeding • Cognitive decline preventing informed consent • Pathological conditions interfering with MS evolution • Allergy to NSAIDs or intolerance to azathioprine or IFN **Duration:** 2 years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included** • N randomised: 150 (N = 77 azathioprine, N = 73 interferon-beta products); one participant in each group dropped after randomisation and before receiving any treatment dose • N = 127 followed at two years • N = 127 followed participants at 2 years included in ITT analysis **Phenotype:** RRMS N = 150 (100% of randomised participants) **Diagnostic criteria:** McDonald 2001 **Sex/gender:** N = 99 female participants (66% of randomized participants), N = 51 male participants **Mean age at study entry:** 38.1 (SD 8.9) years (azathioprine group), 36.6 (SD 8.8) years (interferon-beta products group) **Disease duration:** 6.8 years (SD 7.1) (azathioprine group); 5.7 years (SD 5.7) (interferon-beta products group) **EDSS at baseline:** mean 1.9 (0.9 SD) (azathioprine group); 1.9 (0.9 SD) (interferon-beta products group)
Interventions	**Intervention:** N = 77 (N = 62 followed at two years), azathioprine oral target dose of 3 mg/kg/day, individually adjusted to differential white cell counts **Comparator:** N = 73 (N = 65 followed at two years), interferon beta-1b 250 mg sc on alternate days (Betaferon); interferon beta-1a 30 mg im weekly (Avonex); interferon beta-1a 22/44 mg sc thrice weekly (Rebif). The type of interferon was selected by the treating neurologist.
Outcomes	Annualised relapse ratio over 2 years and during the first and second year; proportion of participants with 0, 1 and 2 or more relapses during the first and second year; proportion of participants with corticosteroid-treated relapses over 2 years; time to first relapse after randomisation; proportion of participants with no confirmed disability progression (without an increase of at least one EDSS point confirmed after at least six months over two years); mean EDSS change from baseline to the end of follow-up; number of treatment failures; mean change of the MSQOL-54 scale over two years MRI outcomes at two years: on 122 participants (61 azathioprine, 61 interferon) with baseline MRI available: annualised new T2 lesions rate; number of participants with new gadolinium-enhancing lesions; T2 lesions load at FLAIR sequence in mm^3 Adverse events and serious adverse events (clinical and instrumental); N of participants withdrawing after adverse events over the course of follow-up
Notes	**Funding source:** Italian national drug agency: AIFA (Agenzia Italiana del Farmaco)

Massacesi 2014 *(Continued)*

Conflicts of interest: reported and include employment, honoraria and reimbursement for meeting participation and educational grants from commercial entities with a potential interest in the study (pharmaceutical companies)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...computer generated central randomization list (1:1 ratio), in blocks of four and stratified by disability score"
Allocation concealment (selection bias)	Low risk	Although the authors do not describe the method of allocation, participants were randomised and allocated centrally, therefore the risk of bias is reasonably low.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Participants and treating neurologists were aware of treatment.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Patients were assessed by...a blinded examining neurologist at their centers. Brain MRI images were centrally analyzed by two blinded independent experts..."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Unbalanced dropout and discontinuation rates at 2 years (both higher in azathioprine group) Quote: "As this event may have diluted true differences between treatments, sensitivity analyses, based on two multiple imputation methods, were performed and no difference in the RRAZA/IFN estimate was observed, thus confirming the results obtained in the analysis of patients who completed the follow-up"
Selective reporting (reporting bias)	Low risk	Protocol provided as supporting infomation. Protocol was amended after two years, when 150 participants had been randomised, instead of the 360 initially planned. The trial steering committee agreed to stop recruitment but to continue the study in consideration of its high informative potential despite the change in sample size. Explanations for the lower recruitment rate and new calculations in view of the reduced sample size are provided.
Other bias	Low risk	Study appeared free of other sources of bias

Milanese 1993

Study characteristics

Methods	**Design:** parallel RCT, 2 arms; superiority **Blinding:** double **Number of centres:** 1 centre in Italy **Inclusion criteria** • Two or more relapses in the 2 years preceding the study in patients with relapsing–remitting course, or steady progression of disability by at least one point on EDSS in the last year in patients with progressive or relapsing-progressive course • EDSS score less than 7

Milanese 1993 *(Continued)*

	• No immunosuppressive treatment in the previous year **Exclusion criteria:** not stated **Duration:** 3 years treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included** • N randomised: 40 (N = 19 azathioprine, N = 21 placebo) • N = 33 participants followed at three years **Phenotypes:** RRMS N = 19 (48% of randomised participants); SPMS N = 10 (25% of randomised participants); PPMS N = 11 (27% of randomised participants). **Diagnostic criteria:** McDonald 1977 **Sex/gender:** not reported **Mean age at study entry:** not stated **Disease duration:** mean 92.2 (SD 50.4) months (azathioprine group); 87.8 (SD 44.9) months (placebo group) **EDSS at study entry:** 3.44 (SD 1.68) (azathioprine group), 3.14 (SD 1.15) (placebo group)
Interventions	**Intervention:** N = 19 (N = 14 followed at 3 years), azathioprine 2 mg/kg orally per day; daily dose rounded off (no more than 2.5 mg/kg per day) to allow single tablet administration and improve participants' compliance **Comparator:** N = 21 (N = 19 followed at 3 years), placebo
Outcomes	Number of relapses per year; number of participants with at least one relapse; progression of disability as expressed (mean change in the EDSS at 1, 2 and 3 years from EDSS score at entry); number of participants who remained stable during the study (no deterioration by 1 or more points on the EDSS score if initial score was 5 or below, or by 0.5 or more if the initial EDSS score was 5.5 or above) Adverse events (clinical and instrumental) over follow-up
Notes	**Funding source:** not reported **Conflicts of interest:** not stated **Comment:** placebo (lactose) was supplied in identical form (50 mg tablets) by Wellcome Italia, which also provided the randomisation code.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Patients were allocated to the azathioprine or placebo groups according to a list of random code numbers."
Allocation concealment (selection bias)	Unclear risk	No information provided on the method used to conceal the allocation sequence
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Placebo (lactose) was supplied in identical form (50 mg tablets)"

Milanese 1993 *(Continued)*

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "...the neurological condition was assessed according to Kurtzke's Functional Systems (FS) and EDSS before entry and every 3 months during the 3-year study by the same blinded neurologist, who also recorded the number of relapses".
Incomplete outcome data (attrition bias) All outcomes	Low risk	At 3 years, 48% of patients on placebo and on 35% of those on azathioprine were still followed. Quote: "13 patients (7 azathioprine, 6 placebo) required the double-blind regimen to be interrupted, mostly within a year of starting treatment".
Selective reporting (reporting bias)	Unclear risk	Protocol unavailable
Other bias	Low risk	Study appeared free of other sources of bias

Milanese 2001

Study characteristics	
Methods	**Design:** prospective parallel cohort study, 3 arms **Blindness:** NA **N of centres:** not stated **Inclusion criteria** • Patients with at least two relapses during the previous 2 years • EDSS lower than or equal to 3.5 **Exclusion criteria:** not stated **Duration:** 1 year treatment/follow-up **Intention-to-treat analysis:** not performed
Participants	**N included** • N = 32 (N = 10 azathioprine, N = 11 interferon beta-1b, N = 11 no treatment) • N = 31 participants followed at 1 year **Phenotype:** RRMS N = 32 (100% of included participants) **Diagnostic criteria:** not stated **Sex/gender:** N = 24 female participants (75% of included participants), 8 male participants **Mean age at study entry**: 31.2 (SD 4.9) years (azathioprine group), 33 (SD 6.2) years (interferon beta-1b group), 38 (SD 6.3) years (no treatment group) **Disease duration:** mean 6.95 (SD 6.7) years (azathioprine group), mean 8.3 (SD 5) years (interferon beta-1b group), mean 8.4 (SD 6.8) years (no treatment group) **EDSS at study entry:** azathioprine: 2.32 (SD 0.9) (interferon beta-1b group), 2.35 (SD 0.9) (azathioprine group), 1.83 (SD 1.15) (no treatment group)
Interventions	**Intervention:** N = 10 (N = 10 followed at 1 year), azathioprine (dosage not stated)

Milanese 2001 *(Continued)*

	Comparator: N = 11 (N = 11 followed at 1 year) interferon beta-1b (dosage not stated), N = 11 (N = 10 followed at 1 year) no treatment
Outcomes	N of participants with disability worsening at 12 months N of relapse-free participants at 12 months Physical composite score (MSQOL-54) at 12 months Mental composite score (MSQOL-54) at 12 months Role limitation for emotional reasons (MSQOL-54) at 12 months Hamilton Depression Rating Scale score at 12 months (data not provided)
Notes	**Funding source:** information not provided **Conflicts of interest:** not stated

Putzki 2006

Study characteristics	
Methods	**Design:** retrospective, controlled cohort study, two arms **Blindness:** NA **N of centres:** 1 centre in Germany **Inclusion criteria:** people with MS followed by the Department of Neurology of the University Hospital of Essen (Germany), contacted through a survey involving 592 people **Exclusion criteria:** not stated **Duration:** median 156 months (range 1 to 276 months) treatment/follow-up **Intention-to-treat analysis:** performed
Participants	**N included:** 317 survey responders **Phenotypes:** RRMS N = 166 (52% of participants), PPMS N = 74 (23% of participants), SPMS N = 77 (24% of participants) **Diagnostic criteria:** not stated **Sex/gender:** N = 104 female participants (33% of participants), N = 213 male participants **Median age at study entry:** 40.5 years (azathioprine group), 42.8 years (non-azathioprine group) **Disease duration:** median 127 months (azathioprine group), 94 months (non-azathioprine group) **EDSS at entry:** not stated
Interventions	**Exposure:** N = 81 (N = 81 at the end of follow-up), treatment with azathioprine. median overall cumulative dosage 108 g (range 2-1080 g). Complete blood counts were obtained for all participants in the group exposed to treatment with azathioprine. **Comparator:** N = 236 (N = 236 at the end of follow-up) participants with MS never treated with azathioprine. Treatments other than azathioprine not reported

Putzki 2006 *(Continued)*

Outcomes	Occurrence of haematological malignancy (myelodisplastic syndrome) in azathioprine group, mortality (follow-up durations variable due to study design)
Notes	**Funding source:** not stated **Conflicts of interest:** not stated **Comment:** complete blood counts were obtained only from patients treated with AZA. People with MS in the group not treated with azathioprine may have been treated with other immunosuppressive drugs

Swinburn 1973

Study characteristics	
Methods	**Design:** prospective, controlled cohort study; 2 arms **Blinding:** single-blind (participants unaware of assigned treatment) **N of centres:** not stated **Inclusion criteria** • Clinically definite MS • Able to walk with or without walking aids **Exclusion criteria** • Female sex • Male sex with diabetes mellitus, liver, or renal disease **Duration:** 2 years treatment/follow-up **Intention-to-treat analysis:** not performed
Participants	**N included** • N = 50 participants (N = 24 azathioprine, N = 26 ascorbic acid) • N = 44 participants followed at 2 years **Phenotypes:** RRMS N = 50 (100% of participants) **Diagnostic criteria:** McAlpine 1968 **Sex/gender:** N = 0 female participants, N = 43 male participants **Mean age at study entry:** 38.53 years (azathioprine group), 40.12 years (ascorbic acid group) **Disease duration:** mean 7.71 years (azathioprine group), 7.52 (ascorbic acid group) **DSS at entry:** not stated
Interventions	**Intervention:** N = 24 (N = 19 followed at two years), azathioprine 2.5 mg/Kg **Comparator:** N = 26 (N = 25 followed at two years) ascorbic acid 50 mg
Outcomes	Kurtzke's Disability Status Scale (mean score before and after intervention, data not reported); N of participants with relapses; relapse score; adverse events at 2 years

Swinburn 1973 *(Continued)*

Notes	**Funding source:** Multiple Sclerosis Society of Great Britain and by Wellcome Research Laboratories provoding azathioprine **Conflicts of interest:** not stated, but funding from private philanthropic organisations

AEs: adverse events; **AZA:** azathioprine; **CBC:** complete blood count; **CFS:** chronic fatigue syndrome; **DMT:** disease-modifying therapies; **DSS:** Disability Status Scale; **EDSS:** Kurtzke Expanded Disability Status Scale; **FLAIR:** fluid-attenuated inversion recovery; **h:** hours; **IFN:** interferons; **IgG:** immunoglobulin G; **im:** intramuscular; **ISS:** Illness Severity Score; **MRI:** magentic resonance imaging; **MS:** multiple sclerosis; **MSQOL-54:** Multiple Sclerosis Quality of Life- 54; **N:** number; **NA:** not applicable; **NSAIDS:** non-steroidal anti-inflammatory drugs; **pmoles:** picomoles; **PMS:** progressive multiple sclerosis; **PPMS:** primary progressive multiple sclerosis; **RCT:** randomised control trial; **RMS:** relapsing multiple sclerosis; **RPMS:** relapsing progressive multiple sclerosis; **RRMS:** relapsing remitting multiple sclerosis; **sc:** subcutaneously; **SD:** standard deviation; **SGOT:** aspartate amino transferase; **SGPT:** alanine amino transferase; **SNE:** stochastic neighbor embedding; **SPMS:** secondary progressive multiple sclerosis; **WBC:** white blood count; **UCLA:** University of California, Los Angeles; **USPHS:** US Public Health Service

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Braun Hashemi 2006	Ineligible outcome (no outcome of interest was measured)
Caputo 1987	Ineligible outcome (no outcome of interest was measured)
Cavazzuti 1997	Ineligible outcome (no outcome of interest was measured)
Cendrowski 1971	Ineligible study design (includes partcipants with ineligible treatment duration: less than 12 months)
Ellison 1984	Ineligible outcome (no outcome of interest was measured)
Lhermitte 1984	Ineligible population (includes participants younger than 18 years of age)
Markovic-Plese 2003	Ineligible study design (uncontrolled study)
Mertin 1982	Ineligible comparator (combination treatment with drugs other than azathioprine that are not present in all arms)
Patzold 1978	Ineligible study design (follow-up less than 12 months)
Patzold 1982	Ineligible outcome (no outcome of interest was measured)
Ravnborg 2009	Ineligible study design (follow-up less than 12 months)
Ring 1974	Ineligible comparator (combination treatment with drugs other than azathioprine that are not present in all arms)
Rosen 1979	Ineligible outcome (no outcome of interest was measured)
Steck 1990	Ineligible outcome (no outcome of interest was measured)
Zeeberg 1985	Ineligible outcome (no outcome of interest was measured)
Zeeberg 1986	Ineligible outcome (no outcome of interest was measured)

Characteristics of studies awaiting classification *[ordered by study ID]*

Aimard 1978

Methods	
Participants	
Interventions	Azathioprine
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor full text are available. Probably a local hospital journal, but unable to identify archive or contact details.

Ciesielski 1974

Methods	
Participants	
Interventions	Azathioprine
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Confavreux 1980

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text are available

Danielczyk 1973

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Ellison 1981

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text are available

Frick 1971

Methods	
Participants	
Interventions	Azathioprine and anti-lymphocytic globulin
Outcomes	
Notes	Unable to determine eligibilty as neither the abstract nor the full text are available

Frick 1974a

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text is available

Frick 1974b

Methods	
Participants	
Interventions	Azathioprine and antilymphocytic globulin
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text is available

Frick 1977

Methods	
Participants	
Interventions	Azathioprine and antilymphocyte globulin
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Gentile 1972

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text are available

Ghezzi 1989

Methods	Randomised controlled trial
Participants	People with multiple sclerosis
Interventions	Azathioprine
Outcomes	
Notes	This study was included in a previous version of the review (Casetta 2007). It is a conference abstract that the previous review authors were able to obtain but we have not been able to, and consequently, we have have not been able to evaluate it according to current methods.

Göpel 1972

Methods	
Participants	
Interventions	Azathioprine
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text is available

Handouk 2009

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility from the conference abstract and the full text is not available

Hervet 1974

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibilty as neither the abstract nor the full text is available

Hitzchke 1979

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text is available

Lhermitte 1984

Methods	
Participants	
Interventions	Azathioprine
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Schluep 1991

Methods	
Participants	
Interventions	Cyclosporine A
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Wilkerson 1975

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility from the abstract and the full text is not available

Yankov 1980

Methods	
Participants	
Interventions	
Outcomes	
Notes	Unable to determine eligibility as neither the abstract nor the full text is available

Characteristics of ongoing studies *[ordered by study ID]*

EUDRACT 2006-004937-13

Study name	**Full name:** Multicenter randomized controlled study of azathioprine versus interferon beta in relapsing remitting multiple sclerosis **Abbreviated name:** M.A.I.N. trial
Methods	Randomised controlled, single-blinded, parallel group trial
Participants	Relapsing remitting multiple sclerosis **Inclusion criteria:** "age 18-55 years; diagnosis of multiple sclerosis McDonald criteria 2001 with relapsin remitting course; at least 2 exacerbation in the preceding 2 years;Baseline EDSS score of 1 to 5,5; no clinical relapses within 30 days of study entry;effective method of contraception if women with childbearing potential; signed informed patient consent."

EUDRACT 2006-004937-13 *(Continued)*

	Exclusion criteria: "immunomodulatory or immunosoppressive tratments in the preceding 6 mounths; steroid therapy in the last 30 days before study entry; concomitant diseases precluding interferon beta or azathioprine treatment; pregnancy or breastfeeding; inability to give the informed consent; pathological conditions interfering with multiple sclerosis evolution; allergy to paracetamol or known intolerance to azathioprine or interferon beta."
Interventions	**Intervention:** azathiprine, 50 mg **Control:** interferon beta-1a, 22 µg
Outcomes	**Primary outcome:** cumulative relapse count per participant over 2 years
Starting date	2006
Contact information	**Sponsor:** Universita di Firenze, Italy
Notes	www.clinicaltrialsregister.eu/ctr-search/trial/2006-004937-13/IT

NCT03653273

Study name	**Full name:** Disease modifying therapies withdrawal in inactive secondary progressive multiple sclerosis patients older than 50 years **Abbreviated name:** STOP-I-SEP trial
Methods	Randomised, controlled, open label, parallel-group study
Participants	Secondary progressive multiple sclerosis **Inclusion criteria** • "Patients > 50 years old; • Secondary progressive phenotype for at least 3 years; The secondary progressive phenotype will be defined as progressive deterioration of disability not due to relapse, with an increase of at least 1 EDSS point since the beginning of the progressive phase (or 0.5 EDSS point if EDSS score ≥ 5.5). • Disease modifying therapy of MS for at least 3 years (interferon, glatiramer acetate, teriflunomide, dimethyl fumarate, cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, rituximab, ocrelizumab); Both patients with the same DMT or with successive DMTs during 3 years can be included. It is important to note that patients could have been treated with fingolimod or natalizumab 2 or 3 years before inclusion, but not during the year before inclusion; • No evidence of focal inflammatory activity for at least 3 years (no clinical relapse and no gadolinium enhancement on an MRI scan); • EDSS≥3. Concomitant medications with Fampridine are allowed throughout the study, provided they have been introduced at least 1 months before inclusion. Natalizumab and fingolimod during the year before inclusion were excluded because of the risk of recurrence of inflammatory activity or even rebound of inflammatory activity after withdrawal. Both patients with the same DMT or with successive DMTs during 3 years can be included, as for example, cyclophosphamide is used for 1 or 2 years, sometimes followed by mycophenolate mofetil. For Rituximab and Ocrelizumab, inclusion in STOP-I-SEP will be at 6 months from the last infusion to take into account the mode of action of these treatments and their specific administration scheme." **Exclusion criteria**

NCT03653273 *(Continued)*

	• "Patients treated with mitoxantrone or alemtuzumab, during the previous 3 years before inclusion; • Patients treated with natalizumab or fingolimod during the year before inclusion; • Change of disease modifying therapy of MS for less than a year • Other neurological or systemic disease ; • Incapacity to understand or sign the consent form ; • Contraindication to MRI ; • Pregnancy or breast-feeding ; • Patient in another clinical trial • Persons referred to in Articles L. 1121-5 to L. 1121-8 and L. 1122-1-2 of the Public Health Code (eg minors, protected adults, …)."
Interventions	**Intervention:** disease-modifying therapy withdrawal **Control:** disease-modifying therapy continuation
Outcomes	**Primary outcome:** percentage of participants experiencing disability progression (confirmed at 6 months) at 2 years **Secondary outcomes:** time of disability progression, disability progression measured by composite score, disability progression measured by change in a composite disability progression score, disability progression measured by symbol digit modalities test, disability progression measured by change in symbol digit modalities test, percentage of patients with relapse, annualised relapse rate, time of relapses, percentage of patients with brain lesion, percentage of patients with gadolinium enhancing lesion, change in brain volume, percentage of patients with no evidence of disease activity, percentage of patients with no evidence of disease activity, percentage of patients who resume DMT in the treatment withdrawal group, quality of life, Medico economic impact, Incremental Cost Effectiveness Ratio (ICER)
Starting date	24 January 2019
Contact information	**Name:** Dr Anne Kerbrat **Email:** anne.kerbrat@chu-rennes.fr
Notes	clinicaltrials.gov/ct2/show/NCT03653273

NCT04106830

Study name	**Full name:** Clinical and imaging patterns of neuroinflammation diseases in China **Abbreviation name:** CLUE trial
Methods	Prospective cohort study
Participants	Patients with neuroinflammatory and demyelination disease **Inclusion criteria** • "18-60 • Diagnosis of neuroinflammatory and demyelination disease • Availability of demographic and clinical data at the time disease onset • Informed written consent obtained from the patient, and/or patient's parent(s), and/or legal representative. Assent, if old enough to grant, will be obtained from all patients under the age of 18 years" **Exclusion criteria**

NCT04106830 *(Continued)*	• "Patients for whom MRI is contra-indicated • Patients included in an ongoing clinical trial where the product is blinded"
Interventions	**Interventions:** intravenous steroids, azathioprine, mycophenolate mofetil, rituximab
Outcomes	**Primary outcomes:** MRI outcomes **Secondary outcomes:** EDSS, timed 25-foot walk, visual acuity
Starting date	1 January 2019
Contact information	**Name:** Yaou Liu, PhD **Email:** yaouliu80@163.com
Notes	https://clinicaltrials.gov/study/NCT04106830

DMT: disease-modifiying therapy; **EDSS:** Expanded Disability Status Scale; **MRI:** magnetic resonance imaging; **MS:** multiple sclerosis; **PhD:** Doctor of Philosophy

DATA AND ANALYSES

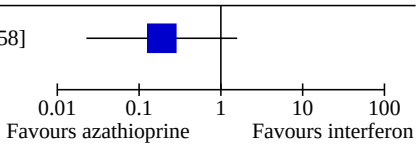
Comparison 1. RRMS, treatment naive, azathioprine versus other DMT (interferon)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Disability: number of participants with disability progression (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.2 Relapse: number of participants with clinical relapse (1- to 2-year follow-up)	2	242	Risk Ratio (M-H, Random, 95% CI)	0.61 [0.43, 0.86]
1.3 Serious adverse events: number of participants with SAEs (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.4 Short-term adverse effects: numbers of participants with gastrointestinal disorders (1- to 2-year follow-up)	2	242	Risk Ratio (M-H, Random, 95% CI)	5.30 [0.15, 185.57]
1.5 Other short-term adverse effects: number of participants with hypersensitivity reactions (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.6 Other long-term adverse effects: number of participants with influenza-like illness (2-year follow-up)	2	242	Risk Ratio (M-H, Random, 95% CI)	0.08 [0.03, 0.24]
1.7 Other long-term adverse effects: number of participants with leukopenia (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.8 Other long-term adverse effects: number of participants with hepatobiliary disorders (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.9 Annualised relapse rate (2-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.10 New or enlarging T2-weighted MRI lesions: number of participants (2-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.11 New gadolinium-enhancing positive T1-weighted MRI lesions: number of participants (2-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.12 Treatment discontinuation due to adverse events: number of participants (1-year follow-up)	2	242	Risk Ratio (M-H, Random, 95% CI)	1.81 [0.83, 3.94]

Analysis 1.1. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 1: Disability: number of participants with disability progression (2-year follow-up)

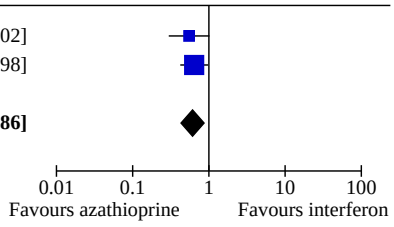
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Random, 95% CI
Massacesi 2014	1	76	5	72	0.19 [0.02 , 1.58]



Analysis 1.2. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 2: Relapse: number of participants with clinical relapse (1- to 2-year follow-up)

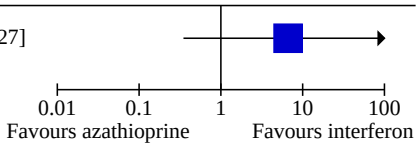
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Weight	Risk Ratio M-H, Random, 95% CI
Etemadifar 2007	11	47	20	47	31.8%	0.55 [0.30 , 1.02]
Massacesi 2014	23	76	34	72	68.2%	0.64 [0.42 , 0.98]
Total		**123**		**119**	**100.0%**	**0.61 [0.43 , 0.86]**
Total events:	34		54			

Test for overall effect: Z = 2.79 (P = 0.005)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.16, df = 1 (P = 0.69); I² = 0%



Analysis 1.3. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 3: Serious adverse events: number of participants with SAEs (2-year follow-up)

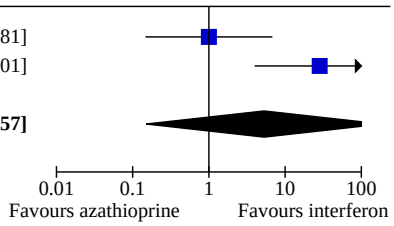
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Random, 95% CI
Massacesi 2014	3	76	0	72	6.64 [0.35 , 126.27]



Analysis 1.4. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 4: Short-term adverse effects: numbers of participants with gastrointestinal disorders (1- to 2-year follow-up)

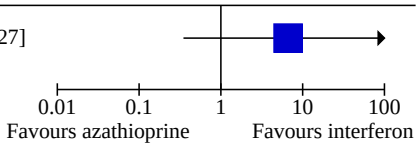
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Weight	Risk Ratio M-H, Random, 95% CI
Etemadifar 2007	2	47	2	47	50.2%	1.00 [0.15 , 6.81]
Massacesi 2014	30	76	1	72	49.8%	28.42 [3.98 , 203.01]
Total		**123**		**119**	**100.0%**	**5.30 [0.15 , 185.57]**
Total events:	32		3			

Test for overall effect: Z = 0.92 (P = 0.36)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 5.60; Chi² = 6.71, df = 1 (P = 0.010); I² = 85%



Analysis 1.5. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 5: Other short-term adverse effects: number of participants with hypersensitivity reactions (2-year follow-up)

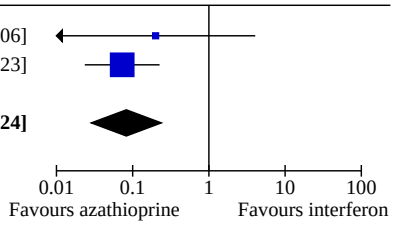
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Random, 95% CI
Massacesi 2014	3	76	0	72	6.64 [0.35 , 126.27]



Analysis 1.6. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 6: Other long-term adverse effects: number of participants with influenza-like illness (2-year follow-up)

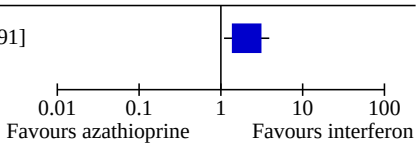
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Weight	Risk Ratio M-H, Random, 95% CI
Etemadifar 2007	0	47	2	47	12.3%	0.20 [0.01 , 4.06]
Massacesi 2014	3	76	39	72	87.7%	0.07 [0.02 , 0.23]
Total		**123**		**119**	**100.0%**	**0.08 [0.03 , 0.24]**
Total events:	3		41			

Test for overall effect: Z = 4.62 (P < 0.00001)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.38, df = 1 (P = 0.54); I² = 0%



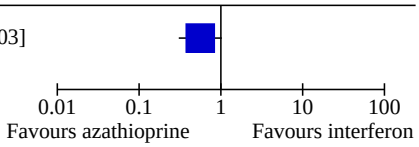
Analysis 1.7. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 7: Other long-term adverse effects: number of participants with leukopenia (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
Massacesi 2014	24	76	11	72	2.07 [1.09 , 3.91]	



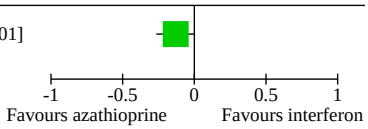
Analysis 1.8. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 8: Other long-term adverse effects: number of participants with hepatobiliary disorders (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
Massacesi 2014	13	76	22	72	0.56 [0.31 , 1.03]	



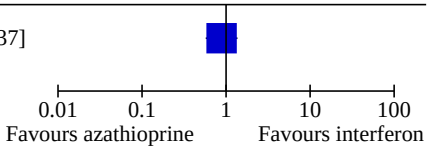
Analysis 1.9. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 9: Annualised relapse rate (2-year follow-up)

Study or Subgroup	Azathioprine Mean	Azathioprine SD	Azathioprine Total	Interferon Mean	Interferon SD	Interferon Total	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
Massacesi 2014	0.26	0.354397	62	0.39	0.42375	65	-0.13 [-0.27 , 0.01]	



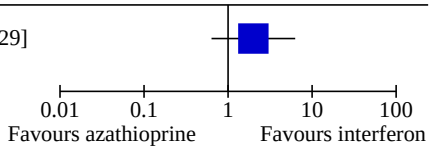
Analysis 1.10. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 10: New or enlarging T2-weighted MRI lesions: number of participants (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI
Massacesi 2014	23	61	26	61	0.88 [0.57 , 1.37]	



Analysis 1.11. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 11: New gadolinium-enhancing positive T1-weighted MRI lesions: number of participants (2-year follow-up)

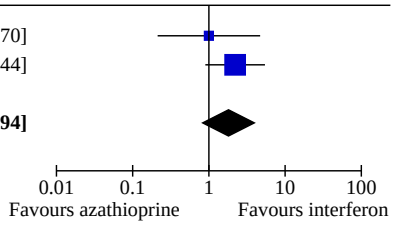
Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Risk Ratio M-H, Fixed, 95% CI
Massacesi 2014	8	61	4	61	2.00 [0.64 , 6.29]



Analysis 1.12. Comparison 1: RRMS, treatment naive, azathioprine versus other DMT (interferon), Outcome 12: Treatment discontinuation due to adverse events: number of participants (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon Events	Interferon Total	Weight	Risk Ratio M-H, Random, 95% CI
Etemadifar 2007	3	47	3	47	25.3%	1.00 [0.21 , 4.70]
Massacesi 2014	14	76	6	72	74.7%	2.21 [0.90 , 5.44]
Total		**123**		**119**	**100.0%**	**1.81 [0.83 , 3.94]**
Total events:	17		9			

Test for overall effect: Z = 1.49 (P = 0.14)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.75, df = 1 (P = 0.39); I² = 0%



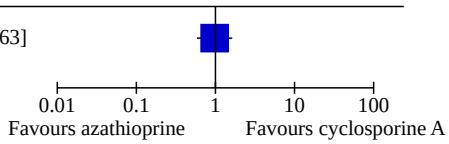
Comparison 2. RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Disability: number of participants with disability progression (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.2 Serious adverse events: number of participants with SAEs (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.3 Short-term adverse effects: number of participants with gastrointestinal disorders (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.4 Other short-term adverse effects: number of participants with hypersensitivity reactions (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.5 Other long-term adverse effects: number of participants with long-term AEs: leukopenia (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.6 Other long-term adverse effects: number of participants with hepatobiliary disorders (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.7 Other long-term adverse effects: number of participants with infections (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.8 Other long-term adverse effects: number of participants with CNS disorders (paresthesia) (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.9 Other long-term adverse effects: number of participants with skin and subcutaneous tissue disorders (hypertrichosis) (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.10 Annualised relapse rate (2-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
2.11 Treatment discontinuation due to adverse events: number of participants (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

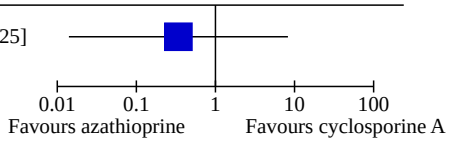
Analysis 2.1. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 1: Disability: number of participants with disability progression (2-year follow-up)

	Azathioprine		Cycloporine A		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	M-H, Random, 95% CI	M-H, Random, 95% CI
Kappos 1988	22	96	23	98	0.98 [0.59 , 1.63]	



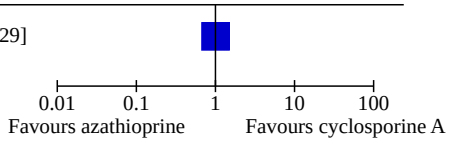
Analysis 2.2. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 2: Serious adverse events: number of participants with SAEs (2-year follow-up)

	Azathioprine		Cyclosporine A		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	M-H, Random, 95% CI	M-H, Random, 95% CI
Kappos 1988	0	96	1	98	0.34 [0.01 , 8.25]	



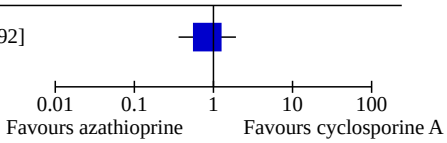
Analysis 2.3. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 3: Short-term adverse effects: number of participants with gastrointestinal disorders (2-year follow-up)

	Azathioprine		Cyclosporine A		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	M-H, Random, 95% CI	M-H, Random, 95% CI
Kappos 1988	53	96	54	98	1.00 [0.78 , 1.29]	



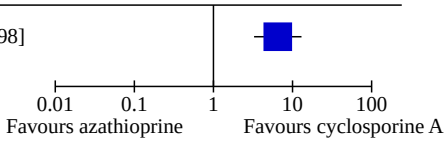
Analysis 2.4. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 4: Other short-term adverse effects: number of participants with hypersensitivity reactions (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Cyclosporine A Events	Cyclosporine A Total	Risk Ratio M-H, Random, 95% CI
Kappos 1988	9	96	11	98	0.84 [0.36 , 1.92]



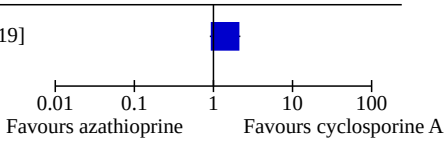
Analysis 2.5. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 5: Other long-term adverse effects: number of participants with long-term AEs: leukopenia (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Cyclosporine A Events	Cyclosporine A Total	Risk Ratio M-H, Random, 95% CI
Kappos 1988	51	96	8	98	6.51 [3.26 , 12.98]



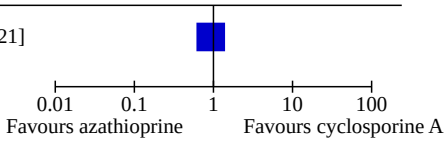
Analysis 2.6. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 6: Other long-term adverse effects: number of participants with hepatobiliary disorders (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Cyclosporine A Events	Cyclosporine A Total	Risk Ratio M-H, Random, 95% CI
Kappos 1988	33	96	24	98	1.40 [0.90 , 2.19]

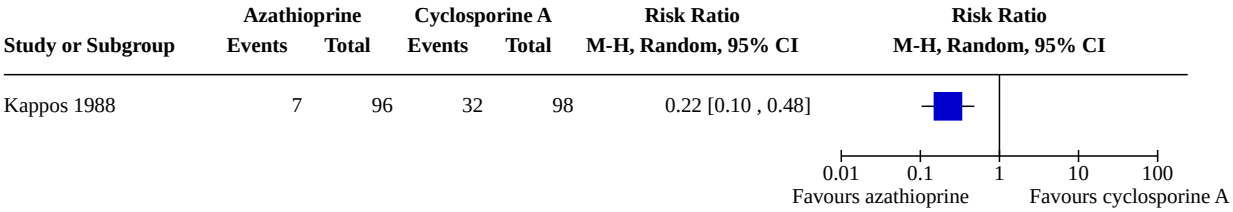


Analysis 2.7. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 7: Other long-term adverse effects: number of participants with infections (2-year follow-up)

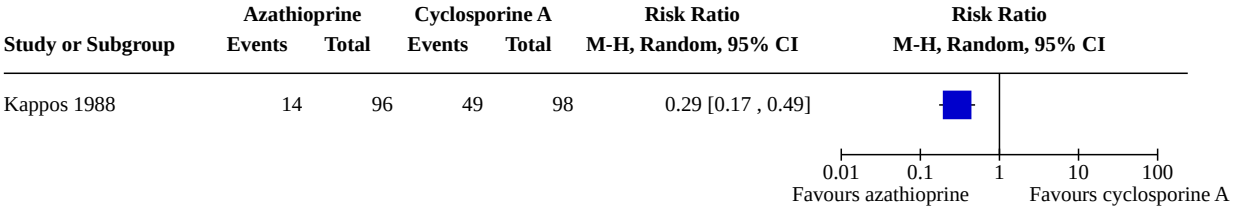
Study or Subgroup	Azathioprine Events	Azathioprine Total	Cyclosporine A Events	Cyclosporine A Total	Risk Ratio M-H, Random, 95% CI
Kappos 1988	48	96	53	98	0.92 [0.71 , 1.21]



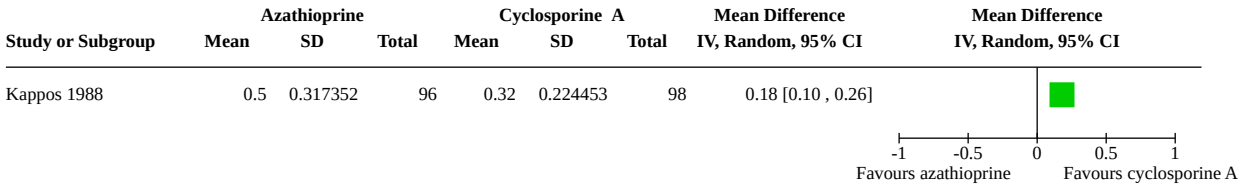
Analysis 2.8. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 8: Other long-term adverse effects: number of participants with CNS disorders (paresthesia) (2-year follow-up)



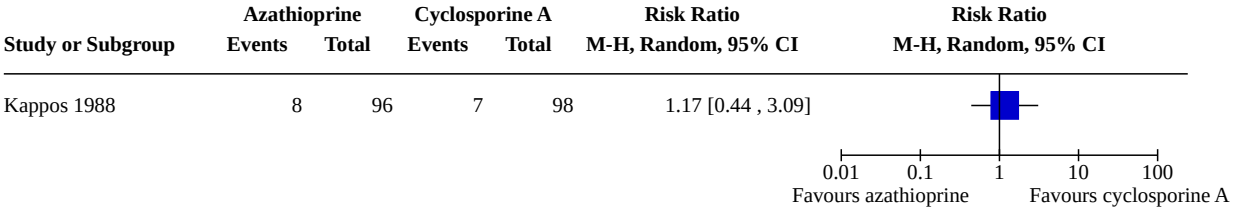
Analysis 2.9. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 9: Other long-term adverse effects: number of participants with skin and subcutaneous tissue disorders (hypertrichosis) (2-year follow-up)



Analysis 2.10. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 10: Annualised relapse rate (2-year follow-up)



Analysis 2.11. Comparison 2: RRMS, treatment naive, azathioprine versus other DMT (cyclosporine A), Outcome 11: Treatment discontinuation due to adverse events: number of participants (2-year follow-up)



Comparison 3. RRMS, treatment naive, azathioprine versus placebo

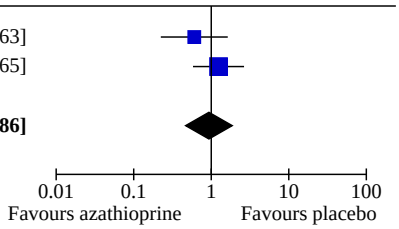
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Disability: number of participants with disability progression (2- to 3-year follow-up)	2	177	Risk Ratio (M-H, Random, 95% CI)	0.93 [0.47, 1.86]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.2 Relapse: number of participants with clinical relapse (2- to 3-year follow-up)	2	177	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.73, 1.12]
3.3 Serious adverse events: number of participants with SAEs (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.4 Short-term adverse effects: number of participants with gastrointestinal disorders (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.5 Long-term adverse effects: number of participants with neoplasms (15-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.6 Mortality: overall number of deaths (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.7 Mortality: overall number of deaths (14-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.8 Other short-term adverse events: number of participants with hypersensitivity reactions (2- to 3-year follow-up)	2	472	Risk Ratio (M-H, Random, 95% CI)	2.32 [1.17, 4.60]
3.9 Other long-term adverse events: number of participants with leukopenia (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.10 Other long-term adverse events: number of participants with hepatobiliary disorders (2- to 3-year follow-up)	2	472	Risk Ratio (M-H, Random, 95% CI)	1.83 [0.37, 9.08]
3.11 Other long-term adverse events: number of participants with infections (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.12 Other long-term adverse events: number of participants with skin and subcutaneous tissue disorders (2-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
3.13 Treatment discontinuation due to adverse events: number of participants (2- to 3-year follow-up)	3	526	Risk Ratio (M-H, Random, 95% CI)	4.85 [0.60, 39.14]

Analysis 3.1. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 1: Disability: number of participants with disability progression (2- to 3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Goodkin 1991	5	30	8	29	39.6%	0.60 [0.22 , 1.63]
Havrdova 2009	12	58	10	60	60.4%	1.24 [0.58 , 2.65]
Total		**88**		**89**	**100.0%**	**0.93 [0.47 , 1.86]**
Total events:	17		18			

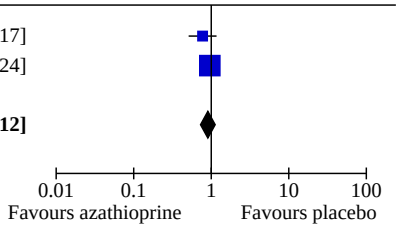
Test for overall effect: Z = 0.20 (P = 0.84)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.06; Chi² = 1.28, df = 1 (P = 0.26); I² = 22%



Analysis 3.2. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 2: Relapse: number of participants with clinical relapse (2- to 3-year follow-up)

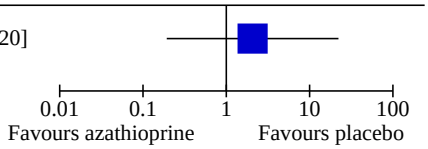
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Goodkin 1991	16	30	20	29	27.3%	0.77 [0.51 , 1.17]
Havrdova 2009	38	58	41	60	72.7%	0.96 [0.74 , 1.24]
Total		**88**		**89**	**100.0%**	**0.90 [0.73 , 1.12]**
Total events:	54		61			

Test for overall effect: Z = 0.91 (P = 0.36)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.76, df = 1 (P = 0.38); I² = 0%



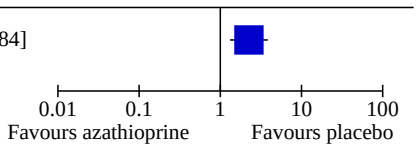
Analysis 3.3. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 3: Serious adverse events: number of participants with SAEs (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Havrdova 2009	2	58	1	60	2.07 [0.19 , 22.20]



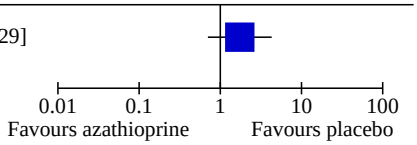
Analysis 3.4. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 4: Short-term adverse effects: number of participants with gastrointestinal disorders (3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	37	174	17	180	2.25 [1.32 , 3.84]



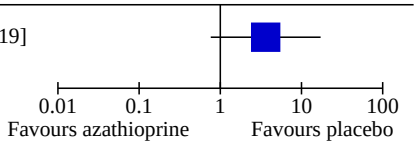
Analysis 3.5. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 5: Long-term adverse effects: number of participants with neoplasms (15-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	12	149	7	151	1.74 [0.70 , 4.29]



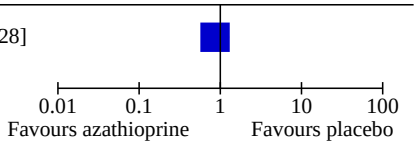
Analysis 3.6. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 6: Mortality: overall number of deaths (3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	7	174	2	180	3.62 [0.76 , 17.19]



Analysis 3.7. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 7: Mortality: overall number of deaths (14-year follow-up)

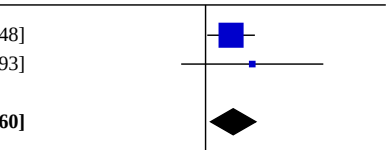
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	34	149	40	151	0.86 [0.58 , 1.28]



Analysis 3.8. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 8: Other short-term adverse events: number of participants with hypersensitivity reactions (2- to 3-year follow-up)

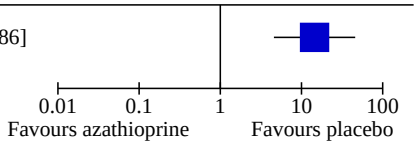
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	21	174	10	180	89.9%	2.17 [1.05 , 4.48]
Havrdova 2009	4	58	1	60	10.1%	4.14 [0.48 , 35.93]
Total		**232**		**240**	**100.0%**	**2.32 [1.17 , 4.60]**
Total events:	25		11			

Test for overall effect: Z = 2.40 (P = 0.02)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.31, df = 1 (P = 0.58); I² = 0%



Analysis 3.9. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 9: Other long-term adverse events: number of participants with leukopenia (3-year follow-up)

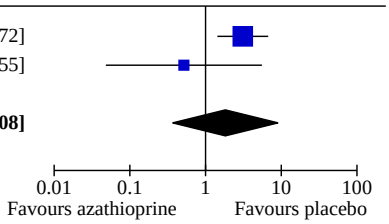
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	42	174	3	180	14.48 [4.57 , 45.86]



Analysis 3.10. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 10: Other long-term adverse events: number of participants with hepatobiliary disorders (2- to 3-year follow-up)

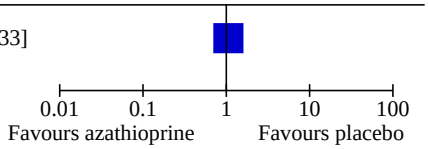
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	24	174	8	180	70.4%	3.10 [1.43 , 6.72]
Havrdova 2009	1	58	2	60	29.6%	0.52 [0.05 , 5.55]
Total		**232**		**240**	**100.0%**	**1.83 [0.37 , 9.08]**
Total events:	25		10			

Test for overall effect: Z = 0.74 (P = 0.46)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.80; Chi² = 1.98, df = 1 (P = 0.16); I² = 50%



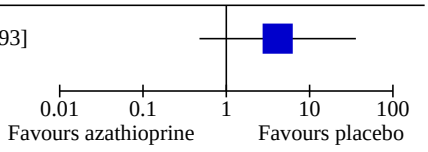
Analysis 3.11. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 11: Other long-term adverse events: number of participants with infections (2-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Havrdova 2009	43	58	42	60	1.06 [0.85 , 1.33]



Analysis 3.12. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 12: Other long-term adverse events: number of participants with skin and subcutaneous tissue disorders (2-year follow-up)

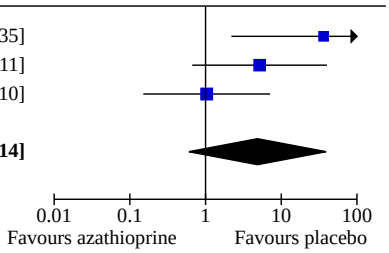
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Havrdova 2009	4	58	1	60	4.14 [0.48 , 35.93]



Analysis 3.13. Comparison 3: RRMS, treatment naive, azathioprine versus placebo, Outcome 13: Treatment discontinuation due to adverse events: number of participants (2- to 3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
British and Dutch 1988	17	174	0	180	27.4%	36.20 [2.19 , 597.35]
Goodkin 1991	6	29	1	25	35.6%	5.17 [0.67 , 40.11]
Havrdova 2009	2	58	2	60	37.0%	1.03 [0.15 , 7.10]
Total		**261**		**265**	**100.0%**	**4.85 [0.60 , 39.14]**
Total events:	25		3			

Test for overall effect: Z = 1.48 (P = 0.14)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 2.10; Chi² = 5.27, df = 2 (P = 0.07); I² = 62%

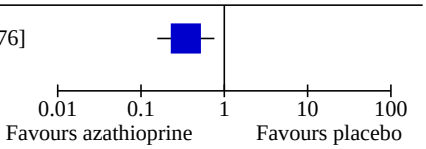


Comparison 4. PMS, treatment naive, azathioprine versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Disability: number of participants with disability progression (2- to 3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.2 Relapse: number of participants with clinical relapse (2- to 3-year follow-up)	2	107	Risk Ratio (M-H, Random, 95% CI)	0.53 [0.35, 0.80]
4.3 Long-term adverse effects: number of participants with neoplasms (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.4 Mortality: overall number of deaths (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.5 Other short-term adverse effects: number of participants with hypersensitivity reactions (2- to 3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.6 Other long-term adverse effects: number of participants with leukopenia (3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.7 Other long-term adverse effects: number of participants with infections (1- to 3-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.8 Treatment discontinuation due to adverse events: number of participants (3-year follow-up)	2	105	Risk Ratio (M-H, Random, 95% CI)	8.73 [1.13, 67.42]

Analysis 4.1. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 1: Disability: number of participants with disability progression (2- to 3-year follow-up)

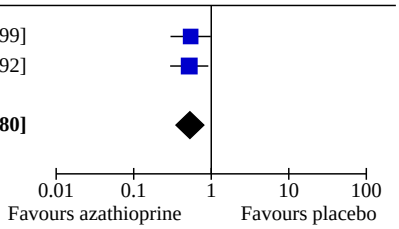
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Milanese 1993	5	19	16	21	0.35 [0.16 , 0.76]



Analysis 4.2. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 2: Relapse: number of participants with clinical relapse (2- to 3-year follow-up)

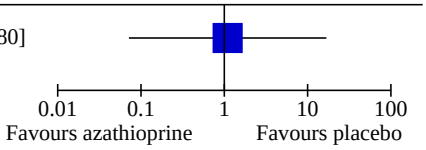
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Ellison 1989	10	33	19	34	47.4%	0.54 [0.30 , 0.99]
Milanese 1993	8	19	17	21	52.6%	0.52 [0.30 , 0.92]
Total		**52**		**55**	**100.0%**	**0.53 [0.35 , 0.80]**
Total events:	18		36			

Test for overall effect: Z = 3.02 (P = 0.003)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.92); I² = 0%



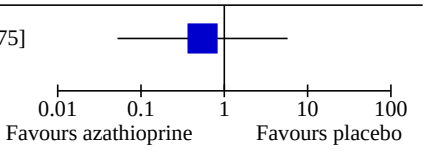
Analysis 4.3. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 3: Long-term adverse effects: number of participants with neoplasms (3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Ellison 1989	1	31	1	34	1.10 [0.07 , 16.80]



Analysis 4.4. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 4: Mortality: overall number of deaths (3-year follow-up)

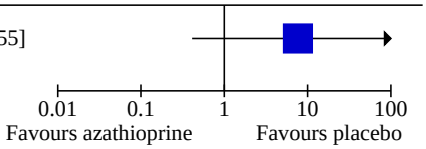
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Ellison 1989	1	31	2	34	0.55 [0.05 , 5.75]



Analysis 4.5. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 5: Other short-term adverse effects: number of participants with hypersensitivity reactions (2- to 3-year follow-up)

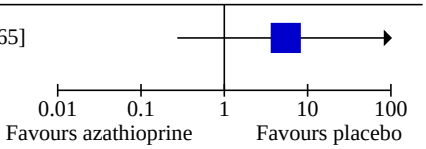
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Ellison 1989	3	31	0	34	7.66 [0.41 , 142.55]

Test for subgroup differences: Not applicable



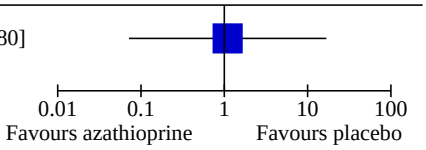
Analysis 4.6. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 6: Other long-term adverse effects: number of participants with leukopenia (3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Ellison 1989	2	31	0	34	5.47 [0.27 , 109.65]



Analysis 4.7. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 7: Other long-term adverse effects: number of participants with infections (1- to 3-year follow-up)

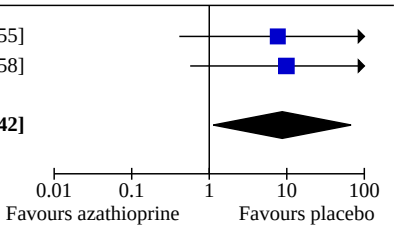
Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Risk Ratio M-H, Random, 95% CI
Ellison 1989	1	31	1	34	1.10 [0.07 , 16.80]



Analysis 4.8. Comparison 4: PMS, treatment naive, azathioprine versus placebo, Outcome 8: Treatment discontinuation due to adverse events: number of participants (3-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Ellison 1989	3	31	0	34	48.9%	7.66 [0.41 , 142.55]
Milanese 1993	4	19	0	21	51.1%	9.90 [0.57 , 172.58]
Total		**50**		**55**	**100.0%**	**8.73 [1.13 , 67.42]**
Total events:	7		0			

Test for overall effect: Z = 2.08 (P = 0.04)
Test for subgroup differences: Not applicable
Heterogeneity: Tau² = 0.00; Chi² = 0.02, df = 1 (P = 0.90); I² = 0%

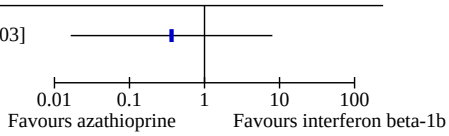


Comparison 5. Non-randomised studies of interventions

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 Disability: number of participants with disability progression (1-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.2 Relapse: number of participants with clinical relapse (1-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.3 Quality-of-life impairment (mental score): mean change in MSQOL-54 mental composite score (1-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
5.4 Other short-term adverse effects: number of people with influenza-like symptoms (1-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.5 Quality-of-life impairment (physical score): mean change in MSQOL-54 physical composite score (1-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
5.6 Disability: number of participants with disability progression (1-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.7 Relapse: number of participants with clinical relapse (1-year follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.8 Quality-of-life impairment (mental score): mean change in MSQOL-54 mental composite score (1-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
5.9 Long-term adverse effects: number of participants with neoplasms (10-year range of follow-up)	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
5.10 Quality-of-life impairment (physical score): mean change in MSQOL-54 physical composite score (1-year follow-up)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected

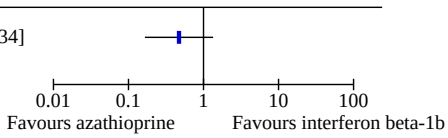
Analysis 5.1. Comparison 5: Non-randomised studies of interventions, Outcome 1: Disability: number of participants with disability progression (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon beta-1b Events	Interferon beta-1b Total	Risk Ratio M-H, Random, 95% CI
Milanese 2001	0	10	1	11	0.36 [0.02 , 8.03]



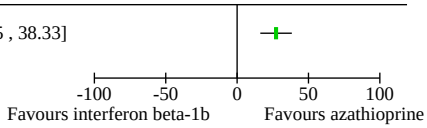
Analysis 5.2. Comparison 5: Non-randomised studies of interventions, Outcome 2: Relapse: number of participants with clinical relapse (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon beta-1b Events	Interferon beta-1b Total	Risk Ratio M-H, Random, 95% CI
Milanese 2001	3	10	7	11	0.47 [0.17 , 1.34]



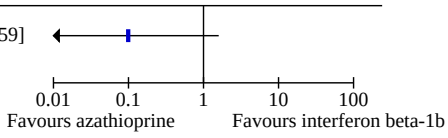
Analysis 5.3. Comparison 5: Non-randomised studies of interventions, Outcome 3: Quality-of-life impairment (mental score): mean change in MSQOL-54 mental composite score (1-year follow-up)

Study or Subgroup	Azathioprine Mean	Azathioprine SD	Azathioprine Total	Interferon beta-1b Mean	Interferon beta-1b SD	Interferon beta-1b Total	Mean Difference IV, Random, 95% CI
Milanese 2001	21.25	11.9	10	-6.04	13.9	11	27.29 [16.25 , 38.33]



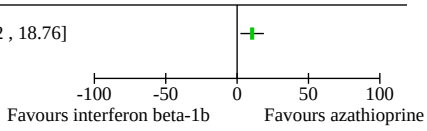
Analysis 5.4. Comparison 5: Non-randomised studies of interventions, Outcome 4: Other short-term adverse effects: number of people with influenza-like symptoms (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	Interferon beta-1b Events	Interferon beta-1b Total	Risk Ratio M-H, Random, 95% CI
Milanese 2001	0	10	5	11	0.10 [0.01 , 1.59]



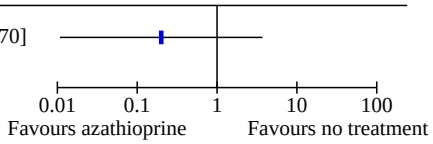
Analysis 5.5. Comparison 5: Non-randomised studies of interventions, Outcome 5: Quality-of-life impairment (physical score): mean change in MSQOL-54 physical composite score (1-year follow-up)

Study or Subgroup	Azathioprine Mean	Azathioprine SD	Azathioprine Total	Interferon beta-1b Mean	Interferon beta-1b SD	Interferon beta-1b Total	Mean Difference IV, Random, 95% CI
Milanese 2001	7.9	9.9	10	-2.64	9.26	11	10.54 [2.32 , 18.76]



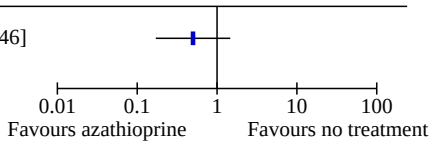
Analysis 5.6. Comparison 5: Non-randomised studies of interventions, Outcome 6: Disability: number of participants with disability progression (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	No treatment Events	No treatment Total	Risk Ratio M-H, Random, 95% CI
Milanese 2001	0	10	2	10	0.20 [0.01 , 3.70]



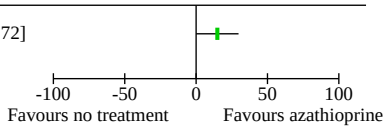
Analysis 5.7. Comparison 5: Non-randomised studies of interventions, Outcome 7: Relapse: number of participants with clinical relapse (1-year follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	No treatment Events	No treatment Total	Risk Ratio M-H, Random, 95% CI
Milanese 2001	3	10	6	10	0.50 [0.17 , 1.46]



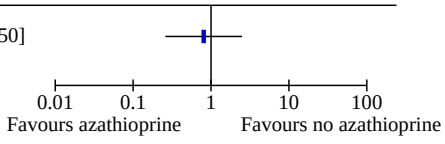
Analysis 5.8. Comparison 5: Non-randomised studies of interventions, Outcome 8: Quality-of-life impairment (mental score): mean change in MSQOL-54 mental composite score (1-year follow-up)

Study or Subgroup	Azathioprine Mean	Azathioprine SD	Azathioprine Total	No treatment Mean	No treatment SD	No treatment Total	Mean Difference IV, Random, 95% CI
Milanese 2001	21.25	11.9	10	6.37	21.8	11	14.88 [0.04 , 29.72]

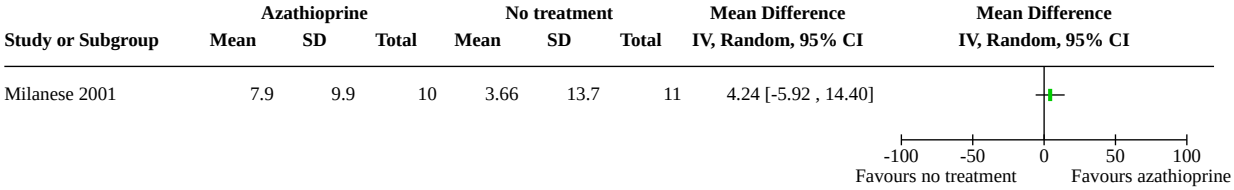


Analysis 5.9. Comparison 5: Non-randomised studies of interventions, Outcome 9: Long-term adverse effects: number of participants with neoplasms (10-year range of follow-up)

Study or Subgroup	Azathioprine Events	Azathioprine Total	No azathioprine Events	No azathioprine Total	Risk Ratio M-H, Random, 95% CI
Amato 1993	5	219	7	247	0.81 [0.26 , 2.50]



Analysis 5.10. Comparison 5: Non-randomised studies of interventions, Outcome 10: Quality-of-life impairment (physical score): mean change in MSQOL-54 physical composite score (1-year follow-up)



ADDITIONAL TABLES

Table 1. Assessment of adverse events monitoring

Study	Active versus spontaneous reporting	AEs defined according to international accepted classification	Serious adverse events specification?*
Randomised control trials			
British and Dutch 1988	Active	No	No
Ellison 1989	Active	No	No
Etemadifar 2007	Active	No	No
Goodkin 1991	Active	No	No
Havrdova 2009	Active	Partly (MedDRA preferred terms)	No. Authors mention “severe adverse events” in three participants (depression-suicide; acute tonsillitis, injection-site inflammation) but provide no a priori definition of SAE
Kappos 1988	Active	No	No
Massacesi 2014	Active	Yes (NCI-CTC)	Yes – likely correlated with treatment: abnormal blood chemistry tests (leukopenia, lymphocytopenia, red blood cell reduction, haemoglobin reduction, thrombocytopenia and other abnormal blood count); fever
Milanese 1993	Active	No	No
Non-randomised studies of interventions			
Amato 1993	Active (in exposed MS)	No	No
Confavreux 1996	Active for cancer symptoms and signs	No	No
Kappos 1990	Not specified	No	No
Milanese 2001	Not specified	No	No

Table 1. Assessment of adverse events monitoring *(Continued)*

Putzki 2006	Not specified	No	No
Swinburn 1973	Active for laboratory alterations (only AZA group). Not further specified	No	No

*Do studies define what they mean by 'serious adverse effects' specifically (and any information on subtypes – short or long term)?
Abbreviations
AEs: adverse events; **AZA:** azathioprine; **MedDRA:** Medical Dictionary for Regulatory Activities; **MS:** multiple sclerosis; **NCI-CTC:** National Cancer Institute-Common Toxicity Criteria; **SAE:** serious adverse events

Table 2. Main characteristics of the participants included in the randomised controlled studies

Study	RRMS	PMS	Age, mean (SD)	Sex (female %)	Duration of disease, mean (SD)	Disability at onset
British and Dutch 1988	67%	33%	39 (SD 8.6) years (azathioprine group); 38 (SD 8.3) years (placebo group)	58% of randomised participants	9 years	DSS: mean 3.69 (SD 1.05) (azathioprine group); mean 3.66 (SD 1.62) (placebo group)
Ellison 1989	-	100%	Not reported	46% of randomised participants	16.7 (SD 10.2) years (azathioprine group); 12.6 (SD 5.6) years (placebo group)	DSS: mean 5.6 (SD 1.2) (azathioprine group); mean 5.5 (SD 1.0) (placebo group)
Etemadifar 2007	100%	-	27.1 (SD 8.8) years (azathioprine group), 28.3 (SD 6.8) years (interferon-beta products group)	79% of randomised participants	Not reported	DSS: mean 1.4 (SD 0.3) (azathioprine group); 1.6 (SD 0.6) (interferon-beta products group)
Goodkin 1991	100%	-	Not reported	61% of randomised participants	6.31 (SD 5.93) years (azathioprine group), 6.24 (SD 8.34) years (placebo group)	EDSS: mean 3.2 (SD 1.2) (azathioprine group); mean 3.7 (SD 1.6) (placebo group)
Havrdova 2009	100%	-	30.9 (SD 7.8) years (azathioprine and interferon beta-1a group), 28.9 (SD 7.1) years (interferon beta-1a and placebo group)	83% of randomised participants	5.5 (SD 5.2) years	EDSS: mean 1.9 (SD 1.1) (azathioprine and interferon beta-1a group); mean 1.8 (SD 0.9) (interferon beta-1a and placebo group)
Kappos 1988	63%	37%	34.7 (SD 9.0) years (azathioprine group), 35.5 (SD 8.4) years (cyclosporine A group)	65% of randomised participants	7.2 (SD 6.9) years (azathioprine group), 6.1 (SD 5.1) years (cyclosporine A group)	EDSS: 0 to 2.5: 44 (46%) (azathioprine group), 45 (46%) (cyclosprine A group); 3 to 4.5: 34 (35%) (azathioprine group), 36 (37%) (cyclosporine A group); 5-6.5: 18 (19%) (azathioprine

Table 2. Main characteristics of the participants included in the randomised controlled studies *(Continued)*

						group), 17 (17%) (cyclosporine A group)
Massacesi 2014	100%	-	38.1 (SD 8.9) years (azathioprine group), 36.6 (SD 8.8) years (interferon-beta products group)	66% of randomised participants	6.8 years (SD 7.1) (azathioprine group); 5.7 years (SD 5.7) (interferon-beta products group)	EDSS: mean 1.9 (0.9 SD) (azathioprine group); 1.9 (SD 0.9) (interferon-beta products group)
Milanese 1993	48%	52%	Not reported	Not reported	92.2 (SD 50.4) months (azathioprine group); 87.8 (SD 44.9) months (placebo group)	EDSS: 3.44 (SD 1.68) (azathioprine group), 3.14 (SD 1.15) (placebo group)

Abbreviations
DSS: Disability Status Scale; **EDSS:** Expanded Disability Status Scale; **PMS:** progressive multiple sclerosis; **RRMS:** relapsing remitting multiple sclerosis; **SD:** standard deviation

Table 3. Main characteristics of the participants included in the non-randomised studies

Study	RRMS	PMS	Age, mean (SD)	Sex (female %)	Duration of disease	Disability at onset
Amato 1993	83%	17%	Reported only age at disease onset	66% of included participants	Reported only duration of treatment and first evaluation in study centre	Not reported
Confavreux 1996	80%	20%	Reported only age at disease onset	57% of included participants	"The average time interval from clinical onset of MS to cancer diagnosis was 13.8 ± 8.1 years (median, 14 years; range, 0.1 to 29.1)"	Not reported
Kappos 1990	Not reported	Not reported	Unclear, reported only for subgroups	62% of included participants	Unclear, reported only for subgroups	Unclear, reported only for subgroups
Milanese 2001	100%	-	31.2 (SD 4.9) years (azathioprine group), 33 (SD 6.2) years (interferon beta-1b group), 38 (SD 6.3) years (no treatment group)	75% of included participants	Mean 6.95 (SD 6.7) years (azathioprine group), mean 8.3 (SD 5) years (interferon beta-1b group), mean 8.4 (SD 6.8) years (no treatment group)	EDSS: azathioprine: 2.32 (SD 0.9) (interferon beta-1b group), 2.35 (SD 0.9) (azathioprine group), 1.83 (SD 1.15) (no treatment group)
Putzki 2006	52%	48%	40.5 years (azathioprine group), 42.8 years (non-azathioprine group)	33% of included participants	Median 127 months (azathioprine group), median 94 months (non-azathioprine group)	Not reported

Table 3. Main characteristics of the participants included in the non-randomised studies *(Continued)*

Swinburn 1973	100%	-	38.53 years (azathioprine group), 40.12 years (ascorbic acid group)	0% of included participants	Mean 7.71 years (azathioprine group), mean 7.52 (ascorbic acid group)	Not reported

Abbreviations
EDSS: Expanded Disability Status Scale; **MS:** multiple sclerosis; PMS: progressive multiple sclerosis; **RRMS:** relapsing remitting multiple sclerosis; **SD:** standard deviation

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs)

Study: Amato 1993

Type of study: retrospective, parallel cohort study

Participants: MS (relapsing remitting MS, primary progressive MS, secondary progressive MS) (n = 454)

Intervention: azathioprine (n = 207)

Active control intervention: other treatment (n = 247)

Outcome timing: range 0.25 to 11.09 years

Outcome assessed

1. Number of participants with long term adverse events - cancer (tumours)

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
1	Harm: cancer	Serious[a]	Moderate[b]	Moderate[c]	Moderate[d]	Low[e]	Serious[f]	Moderate[g]	**SERIOUS**

Abbreviations

MS: multiple sclerosis; **n:** number; **RR:** relapsing remitting

Explanatory footnotes

[a]Only adjustment for age. No information on other factors or previous treatments

[b]Start of follow-up and start of intervention do not coincide for all participants; the authors use appropriate methods to adjust for the bias

[c]Intervention status defined retrospectively

[d]Participants and personnel were aware of the intervention and no information on deviations (co-interventions). All participants were included in the analysis.

[e]All participants included in the analysis

[f]Prone to bias due to lack of blind outcome assessment

[g]Protocol unavailable but selection of the reported result unlikely

Study: Confavreux 1996

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

Type of study: case-control study

Participants: cases: MS with cancer (n = 23); controls: matched MS without cancer (n = 69)

Intervention: azathioprine (14 cases, 34 controls)

Control Intervention: no azathioprine

Outcome timing: range 0 to 21.3 years

Outcome assessed

1. Number of participants with long-term adverse events - cancer (OR 1.7, 95% CI 0.6 to 4.6)

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
1	Harm: cancer	Critical[a]	Serious[b]	Serious[c]	Moderate[d]	Serious[e]	Serious[f]	Moderate[g]	**CRITICAL**

Abbreviations

CI: confidence interval; **MS:** multiple sclerosis; **n:** number; **OR:** odds ratio

Explanatory footnotes

[a]Conditional regressions to consider matching variables. No information on other potential confounders or previous treatments

[b]Start of follow-up and start of intervention are unlikely to coincide for most participants.

[c]The interventions were not well-defined at the individual level.

[d]Investigators were not aware of the intervention and no information on deviations (co-interventions).

[e]Analysis is unlikely to have removed the risk of bias due to missing data.

[f]Investigators were not aware of the intervention when matching; prone to bias due to lack of blind exposure assessment

[g]Protocol unavailable but selection of the reported result unlikely

Study: Kappos 1990

Type of study: matched case-control study

Participants: MS (n = 79; relapsing remitting MS; primary progressive MS; secondary progressiveMS)

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

Intervention: azathioprine (n = 41)

Active control intervention: no immunosuppression (treatment) (n = 38)

Outcome timing: 10 years

Outcome assessed

1. Overall number of deaths

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
O1	Harm: deaths	Critical[a]	Serious[b]	Moderate[c]	No Information[d]	Serious[e]	Low[f]	Moderate[g]	**CRITICAL**

Abbreviations

MS: multiple sclerosis; **n:** number; **RR:** relapsing-remitting

Explanatory footnotes

[a]No information on the distribution of confounding factors amongst the included participants (matched pairs not maintained); only counts available

[b]Start of follow-up and start of intervention do not coincide for most participants.

[c]Intervention status is well defined and some aspects of the assignments of intervention status were determined retrospectively.

[d]Participants and personnel were aware of the intervention and no information is reported on whether there was deviation from the intended intervention.

[e]Not clear which participants were included in the 10-year follow-up

[f]The methods of outcome assessment were comparable across intervention groups; and the outcome measure was unlikely to be influenced by knowledge of the intervention received by study participants.

[g]Protocol unavailable but selection of the reported result unlikely

Study: Milanese 2001

Type of study: cohort study

Participants: relapsing remitting MS (n = 32)

Intervention: azathioprine (n = 10)

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

Active control intervention: IFN α 1b (n = 11)

Control intervention: no treatment (n = 11)

Outcome timing: 12 months

Outcomes assessed:

1. Number of participants with sustained disability worsening (0/10 AZA; 1/11 IFN; 2/10 NT)
2. Number of participants with clinical relapses (3/10 AZA; 7/11 IFN; 6/10 NT)
3. QoL impairment: mean change in QoL score
4. Number of participants with adverse events (AEs)

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
1	Benefit: disability	Serious[a]	Low[b]	Moderate[c]	Moderate[d]	Low[e]	Serious[f]	Moderate[g]	**SERIOUS**
2	Benefit: relapses	Serious[a]	Low[b]	Moderate[c]	Moderate[d]	Low[e]	Serious[f]	Moderate[g]	**SERIOUS**
3	Benefit: QoL	Serious[a]	Low[b]	Moderate[c]	Moderate[d]	NI[h]	Serious[f]	Moderate[g]	**SERIOUS**
4	Harm: AEs	Serious[a]	Low[b]	Moderate[c]	Serious[i]	Low[e]	Serious[f]	Moderate[g]	**SERIOUS**

Abbreviations

AE: adverse effects; **AZA:** azathioprine; **IFN:** interferon; **n:** number; **NT:** no treatment; **QoL:** quality of life; **RMS:** relapsing multiple sclerosis

Explanatory footnotes

[a]No adjustment for confounders. Participants were allocated to IFN or AZA according to participant choice. Participants who refused treatment were allocated to the NT group.

[b]Start of follow-up and start of intervention are likely to coincide for most participants.

[c]The interventions were defined; some aspects of the assignment were determined retrospectively.

[d]Participants and personnel were aware of the intervention and no information was provided on deviations (cointerventions). All participants were included in the analysis.

[e]All participants included in the analysis; one participant in the NT group dropped out at 6 months and was excluded

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

[f]Prone to bias due to lack of blind outcome assessment

[g]Protocol unavailable but selection of the reported result unlikely

[h]The number of participants who did not complete the QoL questionnaire and number of non-completed items in the questionnaire were not reported (NI = no information).

[i]Participants and personnel were aware of the intervention and there was no information on deviations (compliance, cessation of the intervention, shifting) and no appropriate analysis to estimate the effect of adhering to the intervention.

Study: Putzki 2006

Type of study: case-control study

Participants: relapsing remitting MS; primary progressive MS; secondary progressiveMS n = 317

Intervention: azathioprine (n = 81)

Active control intervention: not azathioprine (n = 236)

Outcome timing: 0.1 to more than 10 years

Outcome assessed

1. Number of participants with long term adverse events - cancer

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
1	Harm: cancer	Critical[a]	Critical[b]	Serious[c]	Serious[d]	Serious[e]	Critical[f]	Moderate[g]	**CRITICAL**

Abbreviations
MS: multiple sclerosis; **n:** number

Explanatory footnotes

[a]No adjustment for confounders

[b]Only those participants responding to the questionnaire were included.

[c]The interventions were not well-defined at an individual level; there was no information on the treatment choice.

[d]Missing information prevalent

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

[e]It is not possible to know whether all the data were collected.

[f]Participants not reporting neoplasm were not checked.

[g]Protocol unavailable but selection of the reported result unlikely

Study: Swinburn 1973

Type of study: controlled, non-randomised

Participants: adult men with MS (phenotpe not specified)

Intervention: azathioprine (n = 24)

Active control intervention: ascorbic acid (n = 26)

Outcome timing: year 1 and 2

Outcomes assessed

1. Number of participants with new relapses
2. Number of participants with long-term adverse events (leukopenia)
3. Number of participants who withdrew due to adverse events

Outcome	Benefit or harm of intervention	Domains							Overall risk of bias
		Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	
1	Benefit	Critical[a]	Serious[b]	Low[c]	Low[d]	Serious[e]	Serious[f]	Moderate[g]	**CRITICAL**
2	Harm	Critical[a]	Serious[b]	Low[c]	Low[d]	Serious[e]	Serious[f]	Moderate[g]	**CRITICAL**
3	Harm	Critical[a]	Serious[b]	Low[c]	Low[d]	Serious[e]	Serious[f]	Moderate[g]	**CRITICAL**

Abbreviations

AE: adverse effects; **MS:** multiple sclerosis; **n:** number

Explanatory footnotes

[a]Authors did not use any analysis method to control for confounding; no female participants

[b]Selection of participants for the analysis was based on participant characteristics observed after the start of intervention (adverse events)

Table 4. Summary of risk of bias assessment in non-randomised studies of interventions (NRSIs) *(Continued)*

[c]Definition of intervention determined at the start

[d]Deviations that are expected to arise in usual care

[e]No evidence that the result was not biased by missing data

[f]Outcomes were measured based on participant request and assessors were not blinded.

[g]Protocol unavailable

Table 5. Summary of findings table for additional important outcomes: azathioprine as a first-choice treatment compared with interferon for relapsing multiple sclerosis

Patient or population: adults (aged 18 + years) with relapsing multiple sclerosis

Settings: outpatient

Intervention: azathioprine as first choice treatment

Comparison: interferon

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with interferon	Risk difference with azathioprine				
Other short-term adverse events						
Assessed with: number of participants with hypersensitivity reactions **Follow-up:** 2 years	Not estimable (wide CIs) 0 events in the control group	Not estimable (wide CIs) 3 events in the intervention group	**RR 6.64** (0.35 to 126.27)	148 (1 RCT)	⨁⨁◯◯ Low[a]	-
Other long-term adverse events						
Assessed with: number of participants with influenza-like illness **Follow-up:** range from 1 year to 2 years	345 per 1000	**317 fewer per 1000** (334 fewer to 262 fewer)	**RR 0.08** (0.03 to 0.24)	242 (2 RCTs)	⨁⨁◯◯ Low[b]	-
Assessed with: number of participants with blood disorders (leukopenia) **Follow-up:** 2 years	153 per 1000	**163 more per 1000** (14 more to 445 more)	**RR 2.07** (1.09 to 3.91)	148 (1 RCT)	⨁⨁⨁◯ Moderate[c]	-
Assessed with: number of participants with hepatobiliary disorders (increased ALT) levels **Follow-up:** 2 years	306 per 1000	**134 fewer per 1000** (211 fewer to 9 more)	**RR 0.56** (0.31 to 1.03)	148 (1 RCT) [1]	⨁⨁◯◯ Low[d]	-
Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used in any included study
Quality-of-life impairment (physical score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in

Table 5. Summary of findings table for additional important outcomes: azathioprine as a first-choice treatment compared with interferon for relapsing multiple sclerosis *(Continued)*

						any included study
Annualised relapse rate						
Assessed with: number of relapses per person-year at risk **Follow-up:** 24 months	The mean number of relapses per person-year at risk in the control group was **0.39**	The mean number of relapses per person-year at risk in the intervention group was **0.13 lower** (0.27 lower to 0.01 higher)	-	150 (1 RCT)	⊕◯◯◯ Very low[e]	-
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in any included study
New or enlarging T2-weighted MRI lesions						
Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI **Follow-up:** 2 years	426 per 1000	**51 fewer per 1000** (183 fewer to 158 more)	**RR 0.88** (0.57 to 1.37)	122 (1 RCT)	⊕◯◯◯ Very low[f]	-
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI **Follow-up:** 2 years	66 per 1000	**66 more per 1000** (24 fewer to 347 more)	**RR 2.00** (0.64 to 6.29)	122 (1 RCT)	⊕◯◯◯ Very low[f]	-
Treatment discontinuation due to AEs						
Assessed with: dropouts due to AEs **Follow-up:** range from 1 year to 2 years	76 per 1000	**61 more per 1000** (13 fewer to 222 more)	**RR 1.81** (0.83 to 3.94)	242 (2 RCTs)	⊕◯◯◯ Very low[g]	-

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)

Abbreviations

AE: adverse events; **ALT:** alanine transaminase; **ARR:** annualised relapse rate; **AZA:** azathioprine; **CI:** confidence interval; **GAD:** gadolinium; **IFN:** interferon; **MRI:** magnetic resonance imaging; **MS:** multiple sclerosis; **n:** number; **OIS:** optimal information size; **RCT:** randomised control trial; **RR:** risk ratio

[a]Downgraded two levels for imprecision: OIS not met, wide CIs including no difference, no events in control group; not downgraded for risk of bias: study is single blinded (patients aware of treatment), but detection bias on SAEs is unlikely.

[b]Downgrading one level for risk of bias: the study was single blinded (participants aware of treatment). Downgrading one level for imprecision: OIS not met, CIs do not include harm

[c]Downgraded one level for imprecision: OIS not met, CIs do not include benefit

[d]Not downgraded for risk of bias: study was single blinded (participants aware of treatment), but considering that ALT levels are assessed through instrumental test, detection bias is unlikely. Downgrading two levels for imprecision: OIS not met, wide CIs including appreciable benefit and appreciable harm

[e]Downgrading two levels for risk of bias: the study was single blinded (participants aware of treatment), the ARR calculation was based on 127 participants (azathioprine n = 62, interferon n = 65) who completed the 24-month follow-up (incomplete outcome data). Downgrading one level for imprecision: OIS not met

[f]Downgrading one level for risk of bias: baseline MRI was available only for 61 of 65 (IFN) and 61 of 62 (AZA) participants. Downgrading two levels for imprecision: OIS not met and CIs include both appreciable benefit and appreciable harm

[g]Downgrading one level for risk of bias: the study was single blinded (participants aware of treatment). Downgrading two levels for imprecision: OIS not met and CIs include both appreciable benefit and appreciable harm

Table 6. Summary of findings table: azathioprine as a first-choice treatment compared with cyclosporine A for relapsing multiple sclerosis

Patient or population: adults (aged 18 + years) with relapsing multiple sclerosis

Settings: outpatient

Intervention: azathioprine as a first-choice treatment

Comparison: cyclosporine A

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with cyclosporine A	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 2 years	235 per 1000	**5 fewer per 1000** (from 138 fewer to 383 more)	**RR 0.98** (0.59 to 1.63)	194 (1 RCT)	⨁◯◯◯ Very low[a]	-
Relapse						
Assessed with: number of participants with clinical relapse	-	-	-	-	-	Outcome not used in included study
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs **Follow-up:** 2 years	10 per 1000	**7 fewer per 1000** (from 10 fewer to 74 more)	**RR 0.34** (0.01 to 8.25)	194 (1 RCT)	⨁⨁◯◯ Low[b]	-
Quality-of-life impairment (mental score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in included study

Table 6. Summary of findings table: azathioprine as a first-choice treatment compared with cyclosporine A for relapsing multiple sclerosis *(Continued)*

Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea/vomiting **Follow-up:** 2 years	551 per 1000	**0 fewer per 1000** (from 121 fewer to 160 more)	**RR 1.00** (0.78 to 1.29)	194 (1 RCT)	⊕⊕◯◯ Low[c]	-
Long-term adverse events (neoplasms)						
Assessed with: numbers of participants with neoplasms	-	-	-	-	-	Outcome not used in included study
Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Outcome not used in included study
Other short-term adverse events						
Assessed with: number of participants with hypersensitivity reactions **Follow-up:** 2 years	112 per 1000	**18 fewer per 1000** (from 72 fewer to 103 more)	**RR 0.84** (0.36 to 1.92)	194 (1 RCT)	⊕⊕◯◯ Low[d]	-
Other long-term adverse events						
Assessed with: number of participants with blood disorders (leukopenia) **Follow-up:** 2 years	82 per 1000	**450 more per 1000** (from 184 more to 978 more)	**RR 6.51** (3.26 to 12.98)	194 (1 RCT)	⊕⊕⊕◯ Moderate[e]	-
Assessed with: number of participants with hepatobiliary disorders (increased ALT) levels **Follow-up:** 2 years	245 per 1000	**98 more per 1000** (from 24 fewer to 291 more)	**RR 1.40** (0.90 to 2.19)	194 (1 RCT)	⊕⊕◯◯ Low[f]	-
Assessed with: number of participants with infections **Follow-up:** 2 years	541 per 1000	**43 fewer per 1000** (from 157 fewer to 114 more)	**RR 0.92** (0.71 to 1.21)	194 (1 RCT)	⊕⊕◯◯ Low[f]	-
Assessed with: number of participants with CNS disorders (paraesthesia) **Follow-up:** 2 years	327 per 1000	**255 fewer per 1000** (from 294 fewer to 170 fewer)	**RR 0.22** (0.10 to 0.48)	194 (1 RCT)	⊕⊕⊕◯ Moderate[g]	-
Assessed with: number of participants with skin and subcutaneous tissue disorders (hypertrichosis) **Follow-up:** 2 years	500 per 1000	**355 fewer per 1000** (from 415 fewer to 255 fewer)	**RR 0.29** (0.17 to 0.49)	194 (1 RCT)	⊕⊕⊕◯ Moderate[g]	-

Table 6. Summary of findings table: azathioprine as a first-choice treatment compared with cyclosporine A for relapsing multiple sclerosis *(Continued)*

Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used included study
Quality-of-life impairment (physical score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Annualised relapse rate						
Annualised relapse rate **Follow-up:** 2 years	The mean ARR score in the control group was **0.32**	The mean ARR score in the intervention group was **0.18 higher** (from 0.10 to 0.26 higher)	-	194 (1 RCT)	⊕⊕◯◯ Low[h]	-
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in included study
New or enlarging T2-weighted MRI lesions						
Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI	-	-	-	-	-	Outcome not used in included study
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI	-	-	-	-	-	Outcome not used in included study
Treatment discontinuation due to AEs						
Assessed with: number of participants who discontinued treatment due to adverse events **Follow-up:** 2 years	71 per 1000	**12 more per 1000** (from 40 fewer to 149 more)	**RR 1.17** (0.44 to 3.09)	194 (1 RCT)	⊕⊕◯◯ Low[f]	-

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)
Abbreviations

AEs: adverse events; **ALT:** alanine transaminase; **ARR: annualised relapse rate; CI:** confidence interval; **CNS:** central nervous system; **EDSS:** Expanded Disability Status Scale; **GAD:** gadolinium; **MRI:** magnetic resonance imaging; **MS:** multiple sclerosis; **OIS:** optimal information size; **PMS:** progressive multiple sclerosis; **RCT:** randomised control trial; **RR:** risk ratio; **RRMS:** relapsing remitting multiple sclerosis, **SAE:** serious adverse effect

[a]Downgraded two levels for indirectness: 13% of patients with PMS, subgroup analysis not provided, timing of disability worsening confirmation not specified. Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm.

[b]Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm. Zero events in one group. Not downgraded for indirectness: the trial included both patients with progressive relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since there is no reason to think that the undesirable events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS.

[c]Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm. Not downgraded for indirectness: the trial included both patients with progressive relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since there is no reason to think that the undesirable events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS.

[d]Downgraded two levels for imprecision: OIS not met; CIs include both appreciable benefit and appreciable harm

[e]Not downgraded for indirectness: the trial included both patients with progressive relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since there is no reason to think that the undesirable events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. Downgraded one level for imprecision: OIS not met

[f]Not downgraded for indirectness: the trial included both patients with progressive relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since there is no reason to think that the undesirable events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[g]Not downgraded for indirectness: the trial included both patients with progressive relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since there is no reason to think that the undesirable events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. Downgraded one level for imprecision: OIS not met, CIs do not include harm

[h]Downgraded one level for indirectness: 13% of participants with progressive MS; subgroup analysis was not provided. Downgraded one level for imprecision: OIS not met

Table 7. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for relapsing multiple sclerosis

Patient or population: adults (aged 18 + years) with relapsing multiple sclerosis

Settings: outpatient

Intervention: azathioprine as first choice treatment

Comparison: placebo/no DMT

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with no disease-modifying therapy	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** range from 2 years to 3 years	202 per 1000	**14 fewer per 1000** (107 fewer to 174 more)	**RR 0.93** (0.47 to 1.86)	177 (2 RCTs)	⊕◯◯◯ Very low[a]	-
Relapse						

Table 7. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for relapsing multiple sclerosis *(Continued)*

Assessed with: number of participants with clinical relapse **Follow-up:** range 2 years to 3 years	685 per 1000	**68 fewer per 1000** (185 fewer to 83 more)	**RR 0.90** (0.73, 1.12)	177 (2 RCTs)	⊕◯◯◯ Very low[b]	-
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs **Follow-up:** 2 years	17 per 1000	**18 more per 1000** (14 fewer to 353 more)	**RR 2.07** (0.19 to 22.20)	118 (1 RCT)	⊕◯◯◯ Very low[b]	-
Quality-of-life impairment (mental score)						
Assessed with: -number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea/vomiting **Follow-up:** 3 years	94 per 1000	**118 more per 1000** (30 more to 268 more)	**RR 2.25** (1.32 to 3.84)	354 (1 RCT)	⊕⊕⊕◯ Moderate[c]	-
Long-term adverse events (neoplasms)						
Assessed with: number of participants with neoplasms **Follow-up:** 14 years	46 per 1000	**34 more per 1000** (14 fewer to 153 more)	**RR 1.74** (0.70 to 4.29)	300 (1 RCT)	⊕◯◯◯ Very low[d]	-
Mortality						
Assessed with: number of deaths **Follow-up:** 3 years	11 per 1000	**29 more per 1000** (3 fewer to 180 more)	**RR 3.62** (0.76 to 17.19)	354 (1 RCT)	⊕⊕◯◯ Low[e]	-
Assessed with: number of deaths **Follow-up:** 14 years	265 per 1000	**37 fewer per 1000** (111 fewer to 74 more)	**RR 0.86** (0.58 to 1.28)	300 (1 RCT)	⊕◯◯◯ Very low[f]	-
Other short term adverse events						
Assessed with: number of participants with hypersensitivity reactions **Follow-up:** range from 2 years to 3 years	46 per 1000	**60 more per 1000** (from 8 more to 165 more)	**RR 2.32** (1.17 to 4.60)	472 (2 RCTs)	⊕⊕⊕◯ Moderate[g]	-

Table 7. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for relapsing multiple sclerosis *(Continued)*

Other long-term adverse events						
Assessed with: number of participants with leukopenia **Follow-up:** 3 years	17 per 1000	**225 more per 1000** (60 more to 748 more)	**RR 14.48** (4.57 to 45.86)	354 (1 RCT)	⊕⊕⊕◯ Moderate[g]	-
Assessed with: number of participants with hepatobiliary disorders **Follow-up:** range from 2 years to 3 years	42 per 1000	**35 more per 1000** (26 fewer to 337 more)	**RR 1.83** (0.37 to 9.08)	472 (2 RCTs)	⊕◯◯◯ Very low[b]	-
Assessed with: number of participants with infections **Follow-up:** range from 2 years to 3 years	457 per 1000	**27 more per 1000** (69 fewer to 151 more)	**RR 1.06** (0.85 to 1.33)	118 (1 RCT)	⊕◯◯◯ Very low[b]	-
Assessed with: number of participants with subcutaneous tissue disorders **Follow-up:** 2 years	17 per 1000	**52 more per 1000** (9 fewer to 582 more)	**RR 4.14** (0.48 to 35.93)	118 (1 RCT)	⊕◯◯◯ Very low[b]	-
Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used in any included study
Quality-of-life impairment (physical score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Annualised relapse rate						
Assessed with: number of relapses per person-year at risk	-	-	-	-	-	Outcome not used in any included study
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in any included study
New or enlarging T2-weighted MRI lesions						

Table 7. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for relapsing multiple sclerosis *(Continued)*

Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included study
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included study
Treatment discontinuation due to AEs						
Assessed with: number of participants who discontinued due to AEs **Follow-up:** range 2 years to 3 years	11 per 1000	**44 more per 1000** (4 fewer to 433 more)	**RR 4.85** (0.60 to 39.14)	526 (3 RCTs)	⊕◯◯◯ Very low [h]	-

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)

Abbreviations

AEs: adverse events; **CI:** confidence interval; **DMT:** disease modifying therapies; **EDSS:** Expanded Disability Status Scale; **GAD:** gadolinium; **MRI:** magnetic resonance imaging; **MS:** multiple sclerosis; **OIS:** optimal information size; **PMS:** progressive multiple sclerosis; **RCT:** randomised control trial; **RR:** risk ratio; **RRMS:** relapsing remitting multiple sclerosis; **SAEs:** serious adverse events

[a]Downgraded one level for indirectness (disability confirmed at 2 and 3 months, respectively, instead of 6. In one study, azathioprine was administered at a dose of 50 mg/day, one third of the usual dose as monotherapy, and in combination with interferon, which is uncommon in clinical practice). Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[b]Downgraded one level for indirectness (in one study azathioprine was administered at a dose of 50 mg/day, one third of the usual dose as monotherapy, and in combination with interferon, which is uncommon in clinical practice). Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[c]Downgraded one level for imprecision: OIS not met, CIs do not include appreciable benefit

[d]Not downgraded for indirectness: the trial included both participants with progressive and relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with progressive MS are different from those caused amongst people with RRMS. Downgraded one level for selection bias: the estimate includes data from the British component of the total sample (300 out of 354 participants). Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[e]Not downgraded for indirectness: the trial included both participants with progressive and relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with progressive MS are different from those caused amongst people with RRMS. Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[f]Not downgraded for indirectness: the trial included both patients with progressive and relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with progressive MS are different from those caused amongst people with RRMS. Downgraded one level for selection bias: the estimate includes data from the British component of the total sample (300 out of 354 participants). Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[g]Not downgraded for indirectness: the trial included both participants with progressive and relapsing MS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with progressive MS are different from those caused amongst people with RRMS. Downgraded one level for imprecision: OIS not met, CIs do not include appreciable benefit

[h]Downgraded one level for indirectness (in one study azathioprine was administered at a dose of 50 mg/day, one third of the usual dose as monotherapy, and in combination with interferon, which is uncommon in clinical practice). Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm. Downgraded one level for inconsistency (I^2 = 62%)

Table 8. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for progressive multiple sclerosis

Patient or population: adults (aged 18 + years) with progressive multiple sclerosis

Settings: outpatient

Intervention: azathioprine as a first-choice treatment

Comparison: placebo

Outcome	Anticipated absolute effects * (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo	Risk difference with azathioprine				
Disability						
Assessed with: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 3 years	762 per 1000	**495 fewer per 1000** (640 fewer to 183 fewer)	**RR 0.35** (0.16 to 0.76)	40 (1 RCT)	⊕◯◯◯ Very low[a]	-
Relapse						
Assessed with: number of participants with clinical relapse **Follow-up:** 3 years	655 per 1000	**308 fewer per 1000** (425 fewer to 124 fewer)	**RR 0.53** (0.35 to 0.80)	107 (2 RCTs)	⊕◯◯◯ Very low[b]	-
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Outcome not used in any included study
Quality-of-life impairment (mental score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea/vomiting	-	-	-	-	-	Outcome not used in any included study
Long-term adverse events (neoplasms)						
Assessed with: number of participants with neoplasms **Follow-up:** 3 years	29 per 1000	**3 more per 1000** (27	**RR 1.10** (0.07 to 16.80)	65 (1 RCT)	⊕⊕◯◯ Low[c]	-

Table 8. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for progressive multiple sclerosis *(Continued)*

		fewer to 465 more)				
Mortality						
Assessed with: number of deaths **Follow-up:** 3 years	59 per 1000	**26 fewer per 1000** (56 fewer to 279 more)	**RR 0.55** (0.05 to 5.75)	65 (1 RCT)	⊕⊕◯◯ Low[c]	-
Other short-term adverse events						
Assessed with: number of participants with hypersensitivity reactions **Follow-up:** 3 years	Not estimable (wide CIs)	Not estimable (wide CIs)	**RR 7.66** (0.41 to 142.55)	65 (1 RCT)	⊕⊕◯◯ Low[d]	-
Other long-term adverse events						
Assessed with: number of participants with leukopenia **Follow-up:** 3 years	Not estimable (wide CIs)	Not estimable (wide CIs)	**RR 5.47** (0.27 to 109.65)	65 (1 RCT)	⊕⊕◯◯ Low[e]	-
Assessed with: number of participants with infections **Follow-up:** 3 years	29 per 1000	**3 more per 1000** (27 fewer to 465 more)	**RR 1.10** (0.07 to 16.80)	65 (1 RCT)	⊕⊕◯◯ Low[c]	-
Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used in any included study
Quality-of-life impairment (physical score)						
Assessed with: number of participants reporting quality-of-life impairment	-	-	-	-	-	Outcome not used in any included study
Annualised relapse rate						
Assessed with: number of relapses per person-year at risk	-	-	-	-	-	Outcome not used in any included study
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in any included study

Table 8. Summary of findings table: azathioprine as a first-choice treatment compared with placebo for progressive multiple sclerosis *(Continued)*

New or enlarging T2-weighted MRI lesions						
Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included study
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included study
Treatment discontinuation due to AEs						
Assessed with: number of participants discontinuing treatment due to AEs **Follow-up:** 3 years	Not estimable (wide CIs)	Not estimable (wide CIs)	**RR 8.73** (1.13 to 67.42)	105 (2 RCTs)	⨁⨁◯◯ Low[e]	-

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)

Abbreviations

AEs: adverse events; **CI:** confidence interval; **EDSS:** Expanded Disability Status Scale; **MS:** multiple sclerosis; **OIS:** optimal information size; **PMS:** progressive multiple sclerosis; **RCT:** randomised control trial; **RR:** risk ratio; **RRMS:** relapsing remitting multiple sclerosis; **SAEs:** serious adverse events

[a]Downgraded one level for indirectness: in one study, 48% of the sample were people with RRMS and the remaining were PMS, and no subanalysis was provided. Downgraded two levels for imprecision: OIS not met, very small sample

[b]Downgraded one level for risk of bias: in one study, performance bias and detection bias were likely; monitoring neurologists, clinic coordinator and nurses had access to laboratory reports. Downgraded two levels for indirectness: in one study, 48% of the sample were people with relapsing remitting MS, and no subanalysis was provided. In one study, diagnostic criteria applied to define "progressive MS" (Schumacher 1965) are very different from current criteria. Downgraded one level for imprecision: OIS not met. CIs do not include appreciable harm

[c]Not downgraded for indirectness: in one study, 48% of the sample were people with RRMS and the remaining were PMS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm

[d]Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm. Zero events in control group

[e]Not downgraded for indirectness: in one study, 48% of the sample were people with RRMS and the remaining were PMS, but such heterogeneity in the population was deemed as not relevant in terms of indirectness, since it is referred to the safety profile of azathioprine and there is no reason to think that the adverse events caused by azathioprine in people with PMS are different from those caused amongst people with RRMS. Downgraded two levels for imprecision: OIS not met, CIs include both appreciable benefit and appreciable harm. Zero events in control group

Table 9. Summary of findings: NRSIs

Outcome	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with comparator	Risk difference with azathioprine				
Patient or population: people with relapsing-remitting multiple sclerosis						

Table 9. Summary of findings: NRSIs *(Continued)*

Setting: outpatients

Intervention: azathioprine

Comparator: interferon beta-1b

Disability						
Assessed by: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 1 year	91 per 1000	**58 fewer per 1000** (from 89 fewer to 639 more)	**RR 0.36** (0.02 to 8.03)	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[a]	-
Relapse						
Assessed by: number of participants with clinical relapses **Follow-up:** 1 year	636 per 1000	**337 fewer per 1000** (from 528 fewer to 216 more)	**RR 0.47** (0.17 to 1.34)	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[a]	-
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Outcome not used in any included NRSI
Quality of life impairment (mental score)						
Assessed with: mean change in MSQOL-54 mental composite score (0 to 100) **Follow-up:** 1 year	The mean change in MSQOL-54 mental composite score in the control group was **-6.04** (SD 13.09)	The mean change in MSQOL-54 mental composite score (+21.25; SD 11.9) in the intervention group was **27.29 higher** (16.25 higher to 38.33 higher)	-	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[b]	-
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea/vomiting	-	-	-	-	-	Outcome not used in any included NRSI
Long-term adverse events (neoplasms)						
Assessed with: numbers of participants with neoplasms	-	-	-	-	-	Outcome not used in any included NRSI
Mortality						

Table 9. Summary of findings: NRSIs *(Continued)*

Assessed with: overall number of deaths	-	-	-	-	-	Outcome not used in any included NRSI
Other short-term adverse events						
Assessed by: number of participants with influenza-like illness **Follow-up:** 1 year	455 per 1000	**409 fewer per 1000** (from 405 fewer to 268 more)	**RR 0.10** (0.01 to 1.59)	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[a]	-
Other long-term adverse events						
Assessed with: number of participants with leukopenia; infections; disorders of blood/lymphatic; gastrointestinal, hepatobiliary or immune system disorders; skin/subcutaneous disorders; CNS disorders	-	-	-	-	-	Outcome not used in any included NRSI
Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used in any included NRSI
Quality of life impairment (physical score)						
Assessed by: mean change in MSQOL-54 score (0 to 100) **Follow-up:** 1 year	The mean change in MSQOL-54 physical composite score in the control group was **- 2.64** (SD 9.26)	The mean change in MSQOL-54 physical composite score (+7.9; SD 9.9) in the intervention group was **10.54 higher** (2.32 higher to 18.76 higher)	-	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[b]	-
Annualised relapse rate						
Assessed with: number of relapses per person-year at risk	-	-	-	-	-	Outcome not used in any included NRSI
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in any included NRSI
New or enlarging T2-weighted MRI lesions						

Table 9. Summary of findings: NRSIs *(Continued)*

Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included NRSI
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included NRSI
Treatment discontinuation due to AEs						
Assessed with: number of participants discontinuing treatment due to AEs	-	-	-	-	-	Outcome not used in any included NRSI
Patient or population: people with RRMS **Settings:** outpatients **Intervention:** azathioprine **Comparison:** placebo/no treatment						
Disability						
Assessed by: number of participants with an increase of ≥ 1 point of EDSS after ≥ 6 months **Follow-up:** 1 year	200 per 1000	**160 fewer per 1000** (from 198 fewer to 540 more)	**RR 0.20** (0.01 to 3.70)	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[a]	-
Relapse						
Assessed by: number of participants with relapses **Follow-up:** 1 year	600 per 1000	**300 fewer per 1000** (from 498 fewer to 276 more)	**RR 0.50** (0.17 to 1.46)	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[a]	-
Serious adverse events (SAEs)						
Assessed with: number of participants with SAEs	-	-	-	-	-	Outcome not used in any included NRSI
Quality of life impairment (mental score)						
Assessed with: mean change in MSQOL-54 mental composite score (0 to 100) **Follow-up:** 1 year	The mean change in MSQOL-54 mental composite score in the control group was	The mean change in MSQOL-54 mental composite score (+21.25; SD 11.9) in the intervention group was	-	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[b]	-

Table 9. Summary of findings: NRSIs *(Continued)*

	+6.37 (SD 21.8)	**14.88 higher** (0.04 higher to 29.72 higher)				
Short-term adverse events (gastrointestinal disorders)						
Assessed with: number of participants with nausea/vomiting	-	-	-	-	-	Outcome not used in any included NRSI
Long-term adverse events (neoplasms)						
Assessed by: number of participants with neoplasms **Follow-up:** range up to 10 years	28 per 1000	**5 fewer per 1000** (from 21 fewer to 45 more)	**RR 0.81**** (0.26 to 2.50)	454 (1 retrospective parallel cohort study)	⊕◯◯◯ Very low[c]	-
Mortality						
Assessed with: overall number of deaths	-	-	-	-	-	Outcome not used in any included NRSI
Other short-term adverse events						
Assessed by: number of participants with immune system or skin/subcutaneous tissue disorders, or hypersensitivity reactions.	-	-	-	-	-	Outcome not used in any included NRSI
Other long-term adverse events						
Assessed with: number of participants with leukopenia; infections; disorders of blood/lymphatic; gastrointestinal, hepatobiliary or immune system disorders; skin/subcutaneous disorders; CNS disorders	-	-	-	-	-	Outcome not used in any included NRSI
Adverse events						
Assessed with: number of participants with any adverse event	-	-	-	-	-	Outcome not used in any included NRSI
Quality of life impairment (physical score)						
Assessed with: mean change in MSQOL-54 score (0 to 100) **Follow-up:** 1 year	The mean change in MSQOL-54 physical composite score in the control group was **+3.66** (SD 13.7)	The mean change in MSQOL-54 physical composite score (+7.9; SD 9.9) in the intervention group was **4.24 higher** (5.92	-	21 (1 non-randomised controlled cohort study)	⊕◯◯◯ Very low[d]	-

Table 9. Summary of findings: NRSIs *(Continued)*

		lower to 14.40 higher)				
Annualised relapse rate						
Assessed with: number of relapses per person-year at risk	-	-	-	-	-	Outcome not used in any included NRSI
Cognitive decline						
Assessed with: number of participants with cognitive worsening	-	-	-	-	-	Outcome not used in any included NRSI
New or enlarging T2-weighted MRI lesions						
Assessed with: number of participants with new or enlarged T2 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included NRSI
New GAD-enhancing T1-weighted MRI lesions						
Assessed with: number of participants with new GAD-enhancing T1 weighted lesions at MRI	-	-	-	-	-	Outcome not used in any included NRSI
Treatment discontinuation due to AEs						
Assessed with: number of participants discontinuing treatment due to AEs	-	-	-	-	-	Outcome not used in any included NRSI

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)
**Age-adjusted
Abbreviations
AEs: adverse events; **CI:** confidence interval; **CNS:** central nervous system; **EDSS:** Expanded Disability Status Scale; **GAD:** gadolinium; **MS:** multiple sclerosis; **MRI:** magnetic resonance imaging; **MSQOL:** Multiple Sclerosis Quality of Life; **NRSIs:** non-randomised study of interventions; **OIS:** optimal information size; **PMS:** progressive multiple sclerosis; **RMS:** relapsing-remitting multiple sclerosis; **ROBINS-I:** Risk Of Bias In Non-randomised Studies - of Interventions; **RR:** risk ratio; **SAEs**: serious adverse events; **SD:** standard deviation
[a]Downgraded two levels for risk of bias (ROBINS-I). Downgraded two levels for imprecision: OIS not met. CIs include both appreciable benefit and appreciable harm. Few events
[b]Downgraded two levels for risk of bias (ROBINS-I). Downgraded one level for imprecision: small sample size
[c]Not downgraded for indirectness: the study included both participants with progressive and relapsing MS, but we did not consider this heterogeneity in the sample relevant for indirectness, since there is no reason to think that the adverse events caused by azathioprine in people with PMS are different from those caused amongst people with RMS. Downgraded two levels for imprecision: OIS not met. CIs include both appreciable benefit and appreciable harm.
[d]Downgraded two levels for risk of bias (ROBINS-I). Downgraded two levels for imprecision: OIS not met. CIs include both appreciable benefit and appreciable harm.

APPENDICES

Appendix 1. Search strategies

CENTRAL

#22 #18 AND #21 in Trials
#21 {OR #19-#20}
#20 (Azathioprine or Azathioprina or immuran or imuran or imurel or aza):ti,ab,kw
#19 MeSH descriptor: [Azathioprine] explode all trees
#18 {OR #1-#17}
#17 encephalomyelitis:ti,ab,kw
#16 "transverse myelitis":ti,ab,kw
#15 "clinically isolated syndrome":ti,ab,kw
#14 "demyelinating disorder":ti,ab,kw
#13 adem:ti,ab,kw
#12 "demyelinating disease":ti,ab,kw
#11 "devic disease":ti,ab,kw
#10 "optic neuritis":ti,ab,kw
#9 "neuromyelitis optica":ti,ab,kw
#8 "multiple sclerosis":ti,ab,kw
#7 MeSH descriptor: [Myelitis, Transverse] explode all trees
#6 MeSH descriptor: [Encephalomyelitis, Acute Disseminated] explode all trees
#5 MeSH descriptor: [Demyelinating Autoimmune Diseases, CNS] this term only
#4 MeSH descriptor: [Optic Neuritis] explode all trees
#3 MeSH descriptor: [Demyelinating Diseases] this term only
#2 MeSH descriptor: [Multiple Sclerosis, Relapsing-Remitting] explode all trees
#1 MeSH descriptor: [Multiple Sclerosis] this term only

MEDLINE/PubMed

#28 #25 OR #27

(Continued)

#27 #23 NOT#26
#26 "case reports"[Publication Type]
#25 #23 AND #24
#24(randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab]) NOT (animals [mh] NOT humans [mh])
#23 #19 AND #22
#22 #20 OR #21
#21 aza OR imurel OR imuran OR immuran OR azathioprina OR azathioprine
#20 "Azathioprine"[Mesh]
#19 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18
#18 "Multiple Sclerosis"[Mesh:noexp]
#17 "encephalomyelitis"[Title/Abstract]
#16 "transverse myelitis"[Title/Abstract]
#15 "clinically isolated syndrome"[Title/Abstract]
#14 "demyelinating disorder"[Title/Abstract]
#13 adem[Title/Abstract]
#12 "demyelinating disease"[Title/Abstract]
#11 "devic disease"[Title/Abstract]
#10 "optic neuritis"[Title/Abstract]
#9 "neuromyelitis optica"[Title/Abstract]
#8 "multiple sclerosis"[Title/Abstract]
#7 "Myelitis, Transverse"[Mesh]
#6 "Encephalomyelitis, Acute Disseminated"[Mesh]
#5 "Demyelinating Autoimmune Diseases, CNS"[Mesh:noexp]
#4 "Optic Neuritis"[Mesh]
#3 "Demyelinating Diseases"[Mesh:noexp]
#2 "Multiple Sclerosis, Relapsing-Remitting"[Mesh]
#1 "Multiple Sclerosis, Chronic Progressive"[Mesh]

Embase

#31 #29 AND #30
#30 'embase' NOT ('embase' AND 'medline')
#29 #26 OR #28
#28 #22 NOT #27
#27 'case report'/exp
#26 #22 NOT #25
#25 #23 OR #24
#24 (‘animal experiment’/de NOT (‘human experiment’/de OR ‘human’/de))
#23 ((rat:ti,tt OR rats:ti,tt OR mouse:ti,tt OR mice:ti,tt OR swine:ti,tt OR porcine:ti,tt OR murine:ti,tt OR sheep:ti,tt OR lambs:ti,tt OR pigs:ti,tt OR piglets:ti,tt OR rabbit:ti,tt OR rabbits:ti,tt OR cat:ti,tt OR cats:ti,tt OR dog:ti,tt OR dogs:ti,tt OR cattle:ti,tt OR bovine:ti,tt OR monkey:ti,tt OR monkeys:ti,tt OR trout:ti,tt OR marmoset*:ti,tt) AND ‘animal experiment’/de)
#22 #20 AND #21
#21 'crossover procedure':de OR 'double-blind procedure':de OR 'randomized controlled trial':de OR 'single-blind procedure':de OR random*:de,ab,ti OR factorial*:de,ab,ti OR crossover*:de,ab,ti OR ((cross NEXT/1 over*):de,ab,ti) OR placebo*:de,ab,ti OR ((doubl* NEAR/1 blind*):de,ab,ti) OR ((singl* NEAR/1 blind*):de,ab,ti) OR assign*:de,ab,ti OR allocat*:de,ab,ti OR volunteer*:de,ab,ti
#20 #16 AND #19
#19 #17 OR #18
#18 azathioprine:ti,ab OR azatioprina:ti,ab OR immuran:ti,ab OR imuran:ti,ab OR imurel:ti,ab OR aza:ti,ab
#17 'azathioprine'/exp
#16 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15
#15 encephalomyelitis:ab,ti
#14 'transverse myelitis':ab,ti
#13 'clinically isolated syndrome':ab,ti
#12 'demyelinating disorder':ab,ti
#11 adem:ab,ti
#10 'demyelinating disease':ab,ti
#9 'devic disease':ab,ti
#8 'optic neuritis':ab,ti
#7 'neuromyelitis optica':ab,ti

(Continued)

#6 'multiple sclerosis':ab,ti
#5 'transverse myelitis'/exp
#4 'acute disseminated encephalomyelitis'/exp
#3 'optic neuritis'/exp
#2 'demyelinating disease'/de
#1 'multiple sclerosis'/exp

World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP)

(multiple sclerosis OR neuromyelitis optica OR optic neuritis OR devic disease OR demyelinating disease OR demyelinating disorder OR clinically isolated syndrome OR transverse myelitis OR encephalomyelitis)

AND

(Azathioprine OR Azathioprina OR aza OR immuran OR imuran OR imurel)

United States National Institutes of Health clinical trial register (www.clinicaltrials.gov)

multiple sclerosis OR neuromyelitis optica OR optic neuritis OR devic disease OR demyelinating disease OR demyelinating disorder OR clinically isolated syndrome OR transverse myelitis OR encephalomyelitis

AND

Azathioprine OR Azathioprina OR aza OR immuran OR imuran OR imurel

HISTORY

Protocol first published: Issue 7, 2021

CONTRIBUTIONS OF AUTHORS

Conceptualisation of the review: GF, FN
Design of the review: FN, GF, IC, GI
Co-ordination of the review: FN, BR
Search and selection of studies for inclusion in the review: FN, EB, BR, IC
Collection of data for the review: BR, EB, IC, GI, FN
Assessment of the risk of bias in the studies included: FN, EB, IC
Assessment of certainty in the body of evidence: FN, EB
Interpretation of data: FN, BR
Writing of the review: BR, FN, GF, EB
Guarantor of the review: FN

DECLARATIONS OF INTEREST

FN is Joint Co-ordinating Editor of the Cochrane Multiple Sclerosis and Rare Disease of the CNS review group. He was not involved in the editorial process of the review. FN works as an Epidemiologist and Neurologist within the Italian Public Health Service, at an outpatient clinic at the IRCCS Instituto delle Scienze Neurologiche di Bologna.

EB is a neurologist, working at an outpatient clinic at the IRCCS Instituto delle Scienze Neurologiche di Bologna. She is an author of a manuscript on ponesimod for the treatment of relapsing multiple sclerosis, and has received travel and meeting attendance support from Biogen, Roche, and Sanofi Genzyme; personal payments.

BR has worked as the Managing Editor of the Cochrane Multiple Sclerosis and Rare Disease of the CNS review group and is a Managing Editor with the Central Editorial Service. He was not involved in the editorial process of the review.

IC works at the IRCCS San Camillo Hospital, Venice, Italy.

GF is Joint Co-ordinating Editor of the Cochrane Multiple Sclerosis and Rare Disease of the CNS review group; he was not involved in the editorial process of this review. GF was involved with MAIN trial 2014 (Massacesi 2014), funded by the AIFA (Italian Medicines Agency). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript, and GF declares that no competing interests exist. The MAIN trial was approved by ethics committees in the co-ordinating centre (Careggi University Hospital, Ethic Committee, Florence) and in each of the participating centres (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano; Clinica Neurologica, Novara; Università 'La Sapienza', Roma; Policlinico 'G Rodolico' Azienda Ospedaliero-Universitaria, Catania; Clinica Neurologica 2, Genova; Azienda Ospedaliera Universitaria Integrata, Verona; Ospedale Clinicizzato 'Colle Dall'Ara', Chieti; Università di Sassari, Sassari; Università di Napoli, Napoli; Ospedale S Antonio, Padova; Ospedale Civile S Agostino-Estense, Modena; Ospedale Santa Maria, Reggio Emilia; Policlinico Universitario Mater Domini, Catanzaro; Ospedale S Gerardo, Monza; Azienda Ospedaliero-Universitaria S Anna, Ferrara; Ospedali Riuniti, Ancona; Istituto S Raffaele 'G. Giglio', Cefalu; Azienda Ospedaliero San Giovanni Battista, Università di Torino, Torino; Ospedale Sacro Cuore, Negrar; Ospedale Santa Chiara, Trento; Ospedale Regionale, Bolzano; Azienda Ospedaliero-Universitaria Senese, Policlinico 'Le Scotte', Siena; Ospedale 'Misericordia e Dolce', Prato; Università degli Studi di Pisa, Pisa; Policlinico 'G Martino' Messina; Università degli Studi di Palermo, Palermo; Università Cattolica, Policlinico Gemelli, Roma; Dipartimento Neuroriabilitativo ASL CN1, Cuneo; Luigi Gonzaga Hospital, Orbassano Ethics Committees). The MAIN trial adhered to Good Clinical Practice (GCP) guidelines and Declaration of Helsinki.

GI declares that he has no conflicts of interest. GI is a neurologist. He previously worked in the public health system in Italy and is currently working as neurologist and psychiatrist in private practice.

SOURCES OF SUPPORT

Internal sources

- Istituto di Ricovero e Cura a Carattere Scientifico Istituto delle Scienze Neurologiche di Bologna, Azienda USL di Bologna, Italy

  Salary provision (FN, EB, BR)
- Italian Ministry of Health, Italy

  Open access publication funding

External sources

- Multiple Sclerosis International Federation (MSIF), Other

  MSIF, a not-for-profit organisation, provided partial support for the review to the Editorial Base of Cochrane Multiple Sclerosis and Rare Diseases of the CNS, which is hosted by the IRCCS IStituto delle Scienze Neurologiche di Bologna, AUSL Bologna, Italy.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Changes to the review PICO (participants, interventions, comparators, outcomes)

Population

We found a number of studies eligible for inclusion with mixed populations of people with progressive and relapsing forms of multiple sclerosis. To retain the data but clarify its use in the analyses, we adopted a threshold of 50% for the population to be considered as 'progressive' or 'relapsing'.

Interventions

Since we identified more than one disease-modifying therapy comparator in the included studies, we split the comparison 'Azathioprine as a first-choice treatment compared with other disease-modifying therapies for relapsing multiple sclerosis' into two comparisons (and SoF tables) that compared azathioprine in RMS to interferon and cyclosporine A. We clarified in the Types of studies section that we included 'no treatment' as a comparator.

Outcomes

We clarified the prioritisation of outcomes and outcome measures; specifically, when numbers of people with a reduction in quality of life were not available, we looked at the mean change in quality of life score.

We added hypersensitivity reactions to the outcome 'short-term adverse events' after the initial list of the outcomes was refined by the input of the members of a multi-stakeholder guideline development group (including consumers, advisory groups, clinicians and other healthcare professionals with experience in the field of MS). Amongst 'other short-term adverse events', hypersensitivity reactions were judged by the panel as worth being specifically reported as a relevant outcome for both participants and clinicians.

We also clarified that we made the estimates of participants with adverse events and treatment discontinuations for adverse events by taking as denominator the number of participants who took at least one dose of the treatment or control. This choice was consistent with our purpose to assess treatment-specific adverse events and with the definition of adverse event provided in the *Cochrane Handbook for*

Systematic Reviews of Interventions as one for which the causal relation between the intervention and the event is at least a reasonable possibility (Peryer 2023).

Study design

We did not make any changes to the type of studies we included, but we expanded on our rationale for the inclusion of non-randomised studies in the Why it is important to do this review section and the Types of studies section.

Changes to the prioritisation of comparisons

We updated the Summary of findings and assessment of the certainty of the evidence section to reflect our prioritisation of comparisons that are particularly relevant to people with MS, healthcare workers and decision makers. To this effect, we made the following changes to other sections.

- **Why it is important to do this review:** we clarified the relevance of comparing between DMTs, like azathioprine and interferon beta, by adding: “A global survey by the Multiple Sclerosis International Federation involving 89 countries showed that while interferon beta is the most widely used on-label treatment, azathioprine is used off-label to treat MS in 67% of surveyed countries (Laurson-Doube 2021).”
- **Summary of findings and assessment of the certainty of the evidence:** we clarified that the other DMT being compared to is interferon in the list of core summary of findings tables.

Minor changes to other methods

Data extraction and management

This section was updated to note that we piloted the data extraction sheet.

Unit of analysis issues

We updated this section with the following method: "In this situation we calculated an average of the relevant pair-wise comparisons from the study, and a variance for the study, taking into account the correlation between the comparisons."

Dealing with missing data

In dealing with missing data, consistent with what is recommended by the Handbook (Chapter 10.12.3; Deeks 2020) and MECIR (C64), we decided to perform primary analysis following the principles of intention-to-treat analysis as far as possible without any imputation, since arbitrarily imputing the missing data with replacement values and treating these as if they were observed would not have acknowledged uncertainty in the imputed values and results.

Assessment of risk of bias in the included studies

We assessed the risk of bias due to incomplete outcome data by means of the Cochrane risk of bias tool (RoB 1), and the potential role of attrition bias in the results was carefully interpreted in the discussion.

Assessment of heterogeneity

We clarified how we assessed methodological and statistical heterogeneity in the section.

Data synthesis

We updated this section to note that non-randomised studies of interventions judged to be at critical risk of bias were excluded from analysis.

We updated the section to clarify: “We used the inverse variance method and a random-effects model to synthesise continuous outcome measures.”

Summary of findings and assessment of the certainty of the evidence

We updated this section to clarify: “Disagreements were resolved by discussion. Where necessary, a third review author (GF) was consulted.” We also added: “We downgraded up to a maximum of three levels of certainty. For non-randomised studies, we started our certainty assessment at the level of low certainty, and upgraded or downgraded according to GRADE methodology."

Planned methods not used due to lack of data

Sensitivity analysis

We had planned to perform sensitivity analysis to assess the impact of studies judged at high risk of bias in any domain, by removing them from the analyses. However, we did not do this as we judged all included trials to be at high or unclear risk of bias due to lack of details about concealment of allocation and selective reporting in the trial reports.

Funnel plots

We had planned to use contour-enhanced funnel plots to assess the risk of reporting bias, but as we retrieved fewer than 10 RCTs, we were not able to evaluate this possibility. We further clarified in the Assessment of reporting biases section that we compared published results with the protocols of the studies to assess whether there was any selective reporting of outcomes.

INDEX TERMS

Medical Subject Headings (MeSH)

*Azathioprine [adverse effects] [therapeutic use]; *Immunosuppressive Agents [adverse effects] [therapeutic use]; Multiple Sclerosis [drug therapy]; *Multiple Sclerosis, Chronic Progressive [drug therapy]; *Multiple Sclerosis, Relapsing-Remitting [drug therapy]; Quality of Life; *Randomized Controlled Trials as Topic

MeSH check words

Adult; Female; Humans; Male