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Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis (Review)

Timmer A, Patton PH, Chande N, McDonald JWD, MacDonald JK

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Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis.
Cochrane Database of Systematic Reviews 2016, Issue 5. Art. No.: CD000478.
DOI: 10.1002/14651858.CD000478.pub4.

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[Intervention Review]

Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis

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Editorial group: Cochrane IBD Group.
Publication status and date: New search for studies and content updated (no change to conclusions), published in Issue 5, 2016.

Citation: Timmer A, Patton PH, Chande N, McDonald JWD, MacDonald JK. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database of Systematic Reviews* 2016, Issue 5. Art. No.: CD000478. DOI: 10.1002/14651858.CD000478.pub4.

ABSTRACT

Background

Maintenance of remission is a major issue in inflammatory bowel disease. In ulcerative colitis, the evidence for the effectiveness of azathioprine and 6-mercaptopurine for the maintenance of remission is still controversial.

Objectives

To assess the effectiveness and safety of azathioprine and 6-mercaptopurine for maintaining remission of ulcerative colitis.

Search methods

The MEDLINE, EMBASE and Cochrane Library databases were searched from inception to 30 July 2015. Both full randomized controlled trials and associated abstracts were included.

Selection criteria

Randomized controlled trials of at least 12 months duration that compared azathioprine or 6-mercaptopurine with placebo or standard maintenance therapy (e.g. mesalazine) were included.

Data collection and analysis

Two authors independently extracted data using standard forms. Disagreements were solved by consensus including a third author. Study quality was assessed using the Cochrane risk of bias tool. The primary outcome was failure to maintain clinical or endoscopic remission. Secondary outcomes included adverse events and withdrawal due to adverse events. Analyses were performed separately by type of control (placebo, or active comparator). Pooled risk ratios were calculated based on the fixed-effect model unless heterogeneity was shown. The GRADE approach was used to assess the overall quality of evidence for pooled outcomes.

Main results

Seven studies including 302 patients with ulcerative colitis were included in the review. The risk of bias was high in three of the studies due to lack of blinding. Azathioprine was shown to be significantly superior to placebo for maintenance of remission. Fourty-four per cent (51/115) of azathioprine patients failed to maintain remission compared to 65% (76/117) of placebo patients (4 studies, 232 patients; RR 0.68, 95% CI 0.54 to 0.86). A GRADE analysis rated the overall quality of the evidence for this outcome as low due to risk of bias and

imprecision (sparse data). Two trials that compared 6-mercaptopurine to mesalazine, or azathioprine to sulfasalazine showed significant heterogeneity and thus were not pooled. Fifty per cent (7/14) of 6-mercaptopurine patients failed to maintain remission compared to 100% (8/8) of mesalazine patients (1 study, 22 patients; RR 0.53, 95% CI 0.31 to 0.90). Fifty-eight per cent (7/12) of azathioprine patients failed to maintain remission compared to 38% (5/13) of sulfasalazine patients (1 study, 25 patients; RR 1.52, 95% CI 0.66 to 3.50). One small study found that 6-mercaptopurine was superior to methotrexate for maintenance of remission. In the study, 50% (7/14) of 6-mercaptopurine patients and 92% (11/12) of methotrexate patients failed to maintain remission (1 study, 26 patients; RR 0.55, 95% CI 0.31 to 0.95). One very small study compared azathioprine with cyclosporin and found that there was no significant difference between patients failing remission on azathioprine (50%, 4/8) or cyclosporin (62.5%, 5/8) (1 study, 16 patients, RR 0.80 95% CI 0.33 to 1.92). When placebo-controlled studies were pooled with aminosalicylate-comparator studies to assess adverse events, there was no statistically significant difference between azathioprine and control in the incidence of adverse events. Nine per cent (11/127) of azathioprine patients experienced at least one adverse event compared to 2% (3/130) of placebo patients (5 studies, 257 patients; RR 2.82, 95% CI 0.99 to 8.01). Patients receiving azathioprine were at significantly increased risk of withdrawing due to adverse events. Eight per cent (8/101) of azathioprine patients withdrew due to adverse events compared to 0% (0/98) of control patients (5 studies, 199 patients; RR 5.43, 95% CI 1.02 to 28.75). Adverse events related to study medication included acute pancreatitis (3 cases, plus 1 case on cyclosporin) and significant bone marrow suppression (5 cases). Deaths, opportunistic infection or neoplasia were not reported.

Authors' conclusions

Azathioprine therapy appears to be more effective than placebo for maintenance of remission in ulcerative colitis. Azathioprine or 6-mercaptopurine may be effective as maintenance therapy for patients who have failed or cannot tolerate mesalazine or sulfasalazine and for patients who require repeated courses of steroids. More research is needed to evaluate superiority over standard maintenance therapy, especially in the light of a potential for adverse events from azathioprine. This review updates the existing review of azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis which was published in the Cochrane Library (September 2012).

PLAIN LANGUAGE SUMMARY

Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis

Studies of azathioprine and 6-mercaptopurine for maintenance treatment of ulcerative colitis.
Seven studies were reviewed and provide the best evidence we have. Study quality was mostly poor. The studies tested 302 people over the age of eighteen who had ulcerative colitis. The subjects received oral azathioprine or 6-mercaptopurine, placebo (fake pills) or standard maintenance treatment (mesalazine or sulfasalazine). The studies lasted for at least 12 months.

What is ulcerative colitis and could azathioprine and 6-mercaptopurine work?
Ulcerative colitis is a chronic inflammatory disorder of the colon. The most common symptoms of ulcerative colitis are bloody diarrhoea and abdominal pain. Azathioprine and 6-mercaptopurine are thought to reduce inflammation by blocking the immune system.

What did the studies show?
The studies showed that azathioprine was better than placebo for maintenance treatment (i.e. preventing the disease from coming back once the patient has responded to treatment). Fifty-six per cent of patients treated with azathioprine were disease free after one year of treatment compared to 35% of patients who received placebo.

How safe are azathioprine and 6-mercaptopurine?
The drugs were generally well tolerated and side effects occurred infrequently. However, serious side effects such as acute pancreatitis (inflammation of the pancreas that causes severe abdominal pain - a 2% risk) and bone marrow suppression (failure to make normal blood cells - a 4% risk) can occur. Patients taking these drugs should be regularly monitored for evidence of effectiveness and side effects.

What is the bottom line?
Azathioprine may be an effective maintenance treatment for patients who have failed or cannot tolerate mesalazine or sulfasalazine and for patients who require repeated courses of steroids.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Azathioprine versus placebo for maintenance of remission in ulcerative colitis

Azathioprine versus placebo for maintenance of remission in ulcerative colitis

Patient or population: Patients with quiescent ulcerative colitis
Settings: Outpatient
Intervention: Azathioprine or 6-Mercaptopurine versus placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	AZA versus PBO				
Failure to maintain remission	**650 per 1000[1]**	**442 per 1000** (351 to 559)	**RR 0.68** (0.54 to 0.86)	232 (4 studies)	⊕⊕⊖⊖ **low** [2,3]	
Any adverse events	**26 per 1000[1]**	**65 per 1000** (21 to 201)	**RR 2.51** (0.82 to 7.74)	232 (4 studies)	⊕⊖⊖⊖ **very low** [2,4]	
Withdrawal due to adverse event	**0 per 1000[1]**	**0 per 1000** (0 to 0)	**RR 7.00** (0.38 to 128.87)	152 (3 studies)	⊕⊖⊖⊖ **very low** [2,5]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio; **AZA:** azathioprine; **PBO:** placebo

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Control group risk estimates come from control arm of meta-analysis, based on included trials.
[2] Downgraded one level due to high risk of bias in one study in the pooled analysis (single-blind).
[3] Downgraded one level due to sparse data (127 events).
[4] Downgraded two levels due to very sparse data (12 events).
[5] Downgraded two levels due to very sparse data (3 events).

Summary of findings 2. Azathioprine versus sulfasalazine for maintenance of remission in ulcerative colitis

Azathioprine (AZA) versus sulfasalazine for maintenance of remission in ulcerative colitis

Patient or population: Patients with quiescent ulcerative colitis
Settings: Outpatients
Intervention: Azathioprine versus sulfasalazine

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	AZA versus Sulfasalazine				
Failure to maintain remission	**385 per 1000** [1]	**585 per 1000** (254 to 1348)	**RR 1.52** (0.66 to 3.50)	25 (1 study)	⊕⊖⊖⊖ **very low** [2,3]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Control group risk estimates come from control arm of included study.
[2] Downgraded one level due to high risk of bias (open label design).
[3] Downgraded two levels due to very sparse data (12 events) and wide confidence interval.

Summary of findings 3. 6-Mercaptopurine versus 5-ASA for maintenance of remission in ulcerative colitis

6-Mercaptopurine versus 5-ASA for maintenance of remission in ulcerative colitis

Patient or population: Patients with quiescent ulcerative colitis
Settings: Outpatients
Intervention: 6-Mercaptopurine versus 5-ASA

Outcomes	Illustrative comparative risks* (95% CI)	Relative effect (95% CI)	No of Participants	Quality of the evidence	Comments

	Assumed risk	Corresponding risk		(studies)	(GRADE)
	Control	6-MP versus 5-ASA			
Failure to maintain remission	**1000 per 1000** [1]	**530 per 1000** (310 to 900)	**RR 0.53** (0.31 to 0.90)	22 (1 study)	⊕⊖⊖⊖ **very low** [2,3]

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio; **6-MP:** 6-mercaptopurine

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Control group risk estimates come from control arm of included study.
[2] Down-graded one level due to high risk of bias (open label design).
[3] Down-graded two levels due to very sparse data (15 events).

Summary of findings 4. 6-Mercaptopurine versus methotrexate for maintenance of remission in ulcerative colitis

6-Mercaptopurine versus methotrexate for maintenance of remission in ulcerative colitis

Patient or population: Patients with quiescent ulcerative colitis
Settings: Outpatients
Intervention: 6-Mercaptopurine versus methotrexate

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	6-MP versus MTX				
Failure to maintain remission	**917 per 1000** [1]	**504 per 1000** (284 to 871)	**RR 0.55** (0.31 to 0.95)	26 (1 study)	⊕⊕⊖⊖ **very low** [2,3]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **6-MP:** 6 mercaptopurine; **MTX:** methotrexate

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Control group risk estimates come from control arm of included study.
[2] Downgraded one level due to high risk of bias (open label design).
[3] Downgraded two levels due to very sparse data (18 events).

Summary of findings 5. Azathioprine (AZA) versus cyclosporin of remission in ulcerative colitis

Azathioprine (AZA) versus cyclosporin of remission in ulcerative colitis

Patient or population: Patients with quiescent ulcerative colitis
Setting: Outpatients
Intervention: Azathioprine
Comparison: cyclosporin

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with cyclosporin	Risk with Azathioprine				
Failure to maintain remission	625 per 1000[1]	500 per 1000 (206 to 1000)	RR 0.80 (0.33 to 1.92)	16 (1 study)	⊕⊝⊝⊝ **very low** [2,3]	

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

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Control group risk estimates come from control arm of included study.
[2] Downgraded one level due to unclear risk of bias for random sequence generation, allocation concealment, and blinding.

[3] Downgraded two levels due to very sparse data (9 events).

Summary of findings 6. Adverse events across placebo-controlled and aminosalicylate-comparator trials

Adverse events across placebo-controlled and aminosalicylate-comparator trials

Patient or population: Patients with quiescent ulcerative colitis
Setting: Outpatient
Intervention: Azathioprine and 6-mercaptopurine
Comparison: placebo/active therapy

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo	Risk with Adverse events - all trials				
Any adverse event	23 per 1000[1]	65 per 1000 (23 to 185)	RR 2.82 (0.99 to 8.01)	257 (5 studies)	⊕⊖⊖⊖ **very low** [2,3]	
Withdrawal due to adverse event	0 per 1000[1]	0 per 1000 (0 to 0)	RR 5.43 (1.02 to 28.75)	199 (5 studies)	⊕⊖⊖⊖ **very low** [2,4]	
Acute pancreatitis	0 per 1000[1]	0 per 1000 (0 to 0)	RR 4.13 (0.48 to 35.48)	279 (6 RCTs)	⊕⊖⊖⊖ **very low** [2,5]	
Bone marrow suppression	8 per 1000[1]	24 per 1000 (5 to 114)	RR 3.09 (0.64 to 14.83)	257 (5 RCTs)	⊕⊖⊖⊖ **very low** [2,6]	

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

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Control group risk estimates come from control arm of meta-analysis, based on included trials.
[2] Downgraded one level due to high risk of bias in the pooled studies (blinding).
[3] Downgraded two levels due to very sparse data (14 events).

4 Downgraded two levels due to very sparse data (8 events).
5 Downgraded two levels due to very sparse data (3 events).
6 Downgraded two levels due to very sparse data (6 events).

BACKGROUND

Maintenance of remission is a major issue in inflammatory bowel disease. An ideal maintenance therapy would be effective in reducing the occurrence of relapse, be free of adverse events, inexpensive and easy to use (Campieri 2003). Currently, sulfasalazine or mesalazine (5-ASA) are the standard therapy for quiescent ulcerative colitis with good evidence to support their use (Wang 2016). In distal disease, topical preparations are also effective and safe (Marshall 2012). However, there are patients with relapsing disease despite standard maintenance therapy. In addition, some patients do not tolerate 5-ASA or sulfasalazine, or therapy with these drugs is ineffective (Freeman 2012). Azathioprine or 6-mercaptopurine are commonly used in these settings.

The antimetabolites, azathioprine and 6-mercaptopurine, are purine analogues that interfere with nucleic acid metabolism by acting as substrate competitive antagonists, resulting in immunosuppression and reduced cell proliferation (Dubinsky 2004). 6-Mercaptopurine was first synthesized in 1951 and initially used to treat leukaemia. Azathioprine, its S-substituted precursor, was synthesized in 1957. Azathioprine has a longer half life and a different spectrum and perhaps lower level of adverse events than 6-mercaptopurine (Dubinsky 2004), but there are no comparative trials in humans. Onset of action is delayed for up to 3 to 4 months of treatment (Su 2004). Toxicity, the risk for severe bone marrow suppression in particular, is increased in patients with thiopurine-S-methyltransferase (TPMT) deficiency, which occurs in 0.3% (homozygosity, low or absent levels) and 11% (heterozygosity, intermediate levels) of the general population respectively (Colombel 2000; Weinshilboum 1980).

The use of azathioprine for the treatment of quiescent ulcerative colitis was first reported in 1966 (Bowen 1966). A survey conducted by Hilsden 2003 showed that 12% of the patient members of the Crohn's and Colitis Foundation of Canada who are diagnosed with ulcerative colitis are treated with azathioprine or 6-mercaptopurine. Other surveys have shown that 77% of gastroenterologists in Europe and North America, and up to 93% of British consultant gastroenterologists use azathioprine for the treatment of ulcerative colitis (Meuwissen 2000; Stack 1999). The common practice of using azathioprine or 6-mercaptopurine for maintenance of remission in ulcerative colitis, however, is based on limited data (Bressler 2015). Although evidence exists to support the use of azathioprine and 6-mercaptopurine for maintenance of remission in Crohn's disease (Chande 2015), the use of these drugs for maintenance of remission in ulcerative colitis remains controversial.

The first randomized double blind placebo controlled trial on azathioprine for maintenance treatment in ulcerative colitis was reported by Jewell 1974. There have been few studies since, most of them small, with inconsistent results. Evaluating the evidence concerning the safety and efficacy of purine antimetabolites is important, as azathioprine maintenance is usually considered long term treatment (Meuwissen 2000). Major adverse events may occur necessitating regular monitoring (Chouchana 2012). It is estimated that 9 to 25% of patients on azathioprine discontinue treatment due to adverse events (Gearry 2004; Gearry 2005). These include potentially serious adverse events including bone marrow suppression, pancreatitis, hepatotoxicity, lymphoma, and skin cancer (Kotlyar 2015; Long 2012; Present 1989; Wallace 2001). This systematic review is an update of a previously published Cochrane review (Timmer 2012).

OBJECTIVES

The primary objective of the review was to assess the efficacy and safety of azathioprine and 6-mercaptopurine for the maintenance of remission in ulcerative colitis.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials of at least 12 months duration were considered for review. Comparison treatments included placebo, or other active maintenance therapies. Open label studies were also considered.

Types of participants

Patients in whom azathioprine or 6-mercaptopurine were used to treat ulcerative colitis in remission, with or without a preceding period of induction of remission were considered for inclusion. Remission of ulcerative colitis was defined as mild or absent symptoms with complete discontinuation of corticosteroids, irrespective of the use of prophylactic medication, and continuing evidence on sigmoidoscopy of an uninflamed or grade 1 mucosa (Baron 1964). Patients with chronic active disease were not considered for inclusion.

Types of interventions

Randomized controlled trials of oral azathioprine or 6-mercaptopurine used for the treatment of patients with ulcerative colitis in remission were considered for inclusion. This included trials in which these drugs were added to the treatment of patients in remission, withdrawal studies and studies in which there was more than one phase (e.g. active followed by maintenance).

Types of outcome measures

The primary outcome was defined as failure to maintain clinical or endoscopic remission at 12 months from randomization or later, i.e. clinical or endoscopic relapse, or early withdrawal from the study as defined by the investigators. For studies where life table analysis was used the estimated probability of relapse over time was to be examined. Patients failing to achieve clinical remission during an induction phase were considered treatment failures to maintenance therapy in an intention-to-treat approach. Separate analyses were performed excluding these cases.

Secondary outcomes included the occurrence of any adverse event (particularly opportunistic infection, pancreatitis, bone marrow suppression, cancer and death) and withdrawal due to adverse events. Data were extracted to investigate the influence of the dose of azathioprine or 6-mercaptopurine treatment, the duration of previous azathioprine or 6-mercaptopurine treatment (for withdrawal trials), and the effect of other concurrent therapies.

Search methods for identification of studies

We searched MEDLINE (Ovid), EMBASE (Ovid), and the Cochrane Library from inception to 30 July 2015 to identify relevant studies.

Conference proceedings and references were also searched to identify additional studies. The electronic search strategies are reported in Appendix 1.

Data collection and analysis

Evaluation of Included Studies

Trials identified by the search strategy were independently assessed for inclusion by two authors (PP and JKM). Two authors (PP and JKM) independently extracted data from included studies. A third author (NC) was involved for problematic issues. Methodological criteria as well as the results of each study were recorded on standard data forms. All results were tabulated on an intention-to-treat basis. Results excluding treatment failures during an induction period, if present, were also tabulated. The methodological quality of the studies included in this review was assessed using the Cochrane risk of bias tool (Higgins 2011), which involves examining the following study characteristics:

1. Randomization sequence generation;
2. Patient allocation concealment;
3. Blinding;
4. Incomplete outcome data;
5. Selective reporting; and
6. Other sources of bias.

Studies were then judged as being at high, low or unclear risk of bias based on the evidence provided in the available publications (Higgins 2011). Any disagreement between the authors was resolved by consensus.

The GRADE approach was used to assess the overall quality of evidence supporting the outcomes in this review (Guyatt 2008; Schünemann 2011). This approach classifies outcomes as high, moderate, low or very low quality. Data from randomized trials start as high quality evidence. Evidence may be downgraded based on the following criteria:

1. Risk of bias from the studies;
2. Indirect evidence;
3. Inconsistency (i.e. unexplained heterogeneity);
4. Imprecision; and
5. Publication bias.

The overall quality of evidence behind each outcome was determined after considering each of these elements, and categorized as high quality (i.e. further research is very unlikely to change our confidence in the estimate of effect); moderate quality (i.e. further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate); low quality (i.e. further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate); and very low quality (i.e. we are very uncertain about the estimate) (Guyatt 2008; Schünemann 2011). Any disagreements between the authors on the GRADE analysis were resolved by consensus.

Statistical methods:

For each individual study a risk ratio (RR) for the primary outcome (relapse during the study period) along with 95% confidence intervals (95% CI) was calculated based on two by two tables for comparable periods of follow-up (12 months). All patients randomized were included following an intention-to-treat approach, but additional analysis were performed excluding primary treatment failures from studies including an induction period. The presence of heterogeneity among studies was assessed using the chi^2 test, a P value of 0.10 was regarded as statistically significant. The I^2 statistic was used to quantify inconsistency (Higgins 2003). This statistic describes the percentage of the variability in effect estimates that are due to heterogeneity rather than sampling error. A value greater than 50% was considered evidence of substantial heterogeneity. In the presence of significant heterogeneity, pooled analysis was not performed. If homogeneity was likely (I^2 < 0.03), pooled RR with 95% confidence intervals were calculated using a fixed-effect model based on the method by Mantel and Haenszel as a primary analysis.

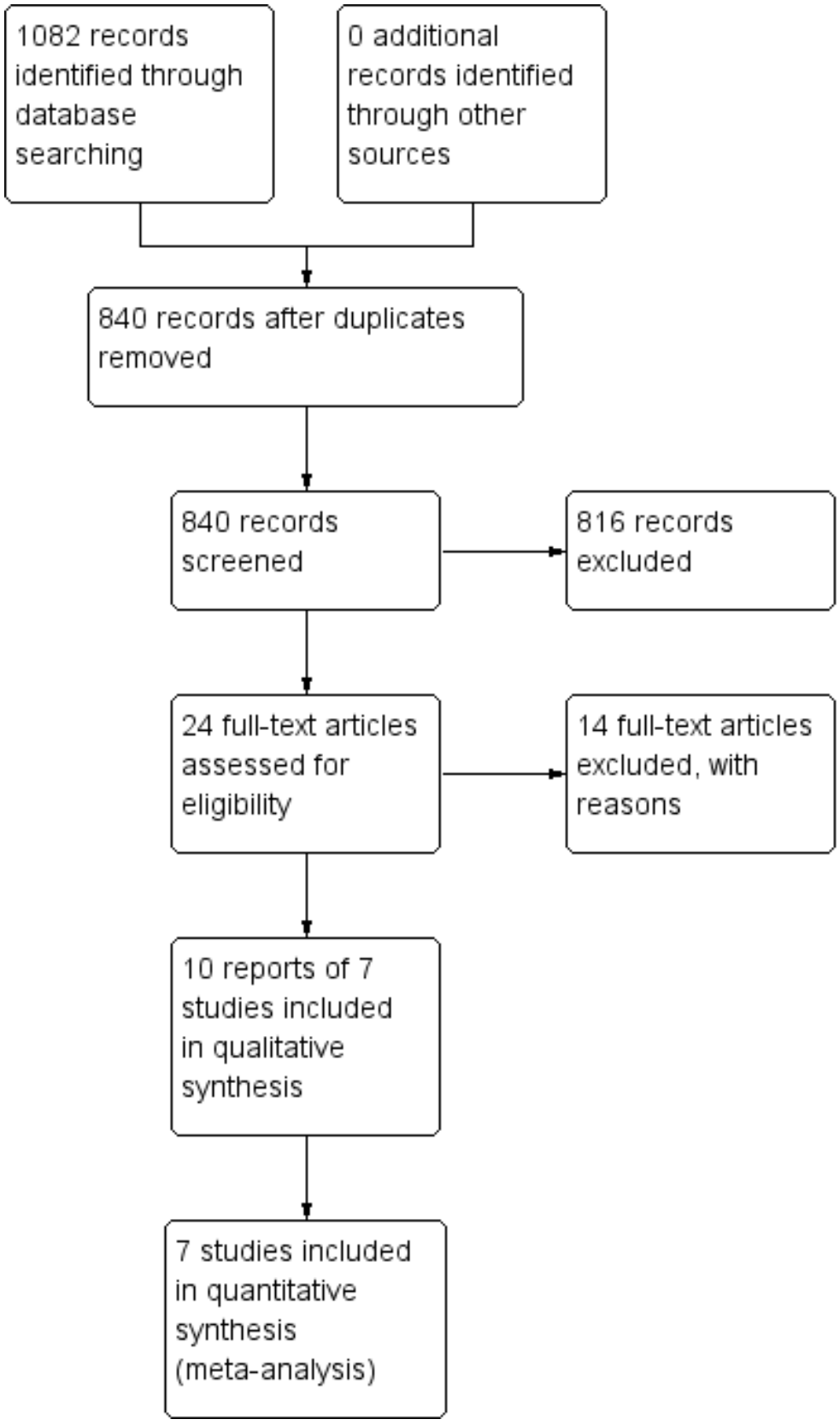
Sensitivity analyses were to be performed to examine the effects of drug dose (corrected for the differential potency of azathioprine and 6-mercaptopurine), type of control intervention (active control, placebo) and study quality (concealed allocation, blinding) on the results of the analysis. Planned analyses also included calculating RR separately for withdrawal studies, studies including an induction period, and studies adding azathioprine to patients already in stable remission.

RESULTS

Description of studies

A literature search conducted on July 30, 2015 identified 1082 records. After duplicates were removed, a total of 840 trials remained for review of titles and abstracts. Two authors (PP and JKM) independently reviewed the titles and abstracts of these trials and 24 studies were selected for full text review (see Figure 1). Fourteen of these studies were excluded (See: Characteristics of excluded studies). Ten reports of 7 studies, including 302 patients, were identified which examined the efficacy of purine antimetabolites compared to placebo or active maintenance therapy in ulcerative colitis (Hawthorne 1992; Jewell 1974; Mate-Jimenez 2000; Paraskeva 2000; Sood 2000; Sood 2002; Sood 2003). The studies used azathioprine in different doses: 2.0 mg/kg/d throughout (Sood 2000), 2.5 mg/kg/d for three months followed by a reduction to 1.5 to 2.0 mg/kg/d (Jewell 1974), 2.5 mg/kg/d throughout (Paraskeva 2000; Sood 2002; Sood 2003) or at variable dosing (Hawthorne 1992). There was only one study examining 6-mercaptopurine at a dose of 1.5 mg/kg/d (Mate-Jimenez 2000). Four studies were placebo controlled (Hawthorne 1992; Jewell 1974; Sood 2000; Sood 2002), three used active comparators (mesalazine and methotrexate - Mate-Jimenez 2000; cyclosporin - Paraskeva 2000; sulfasalazine - Sood 2003). In the placebo controlled study by Sood 2000, all patients received co-medication with 6 g/day sulfasalazine. Co-medication with sulfasalazine or mesalazine was allowed in the study by Hawthorne 1992. In Paraskeva 2000, all patients were allowed tapering steroids and maintenance therapy with 5-ASA.

Figure 1. Study flow diagram.



In all but two of the trials (Hawthorne 1992; Paraskeva 2000), patients were randomized during active disease, and maintenance was preceded by an induction period. A steroid tapering scheme was used at the beginning of all of these trials. In addition, antibiotics were given during the induction period in two studies (Sood 2000; Sood 2002). In contrast, the study by Hawthorne 1992 used a withdrawal design where patients who had been in remission on azathioprine for 6 months or longer before being randomized to withdrawal (i.e. placebo) or maintenance on azathioprine. The study by Paraskeva 2000 used a 6 month treatment phase with either azathioprine or cyclosporin coupled with tapering steroids and 5-ASA treatment before discontinuing all medications.

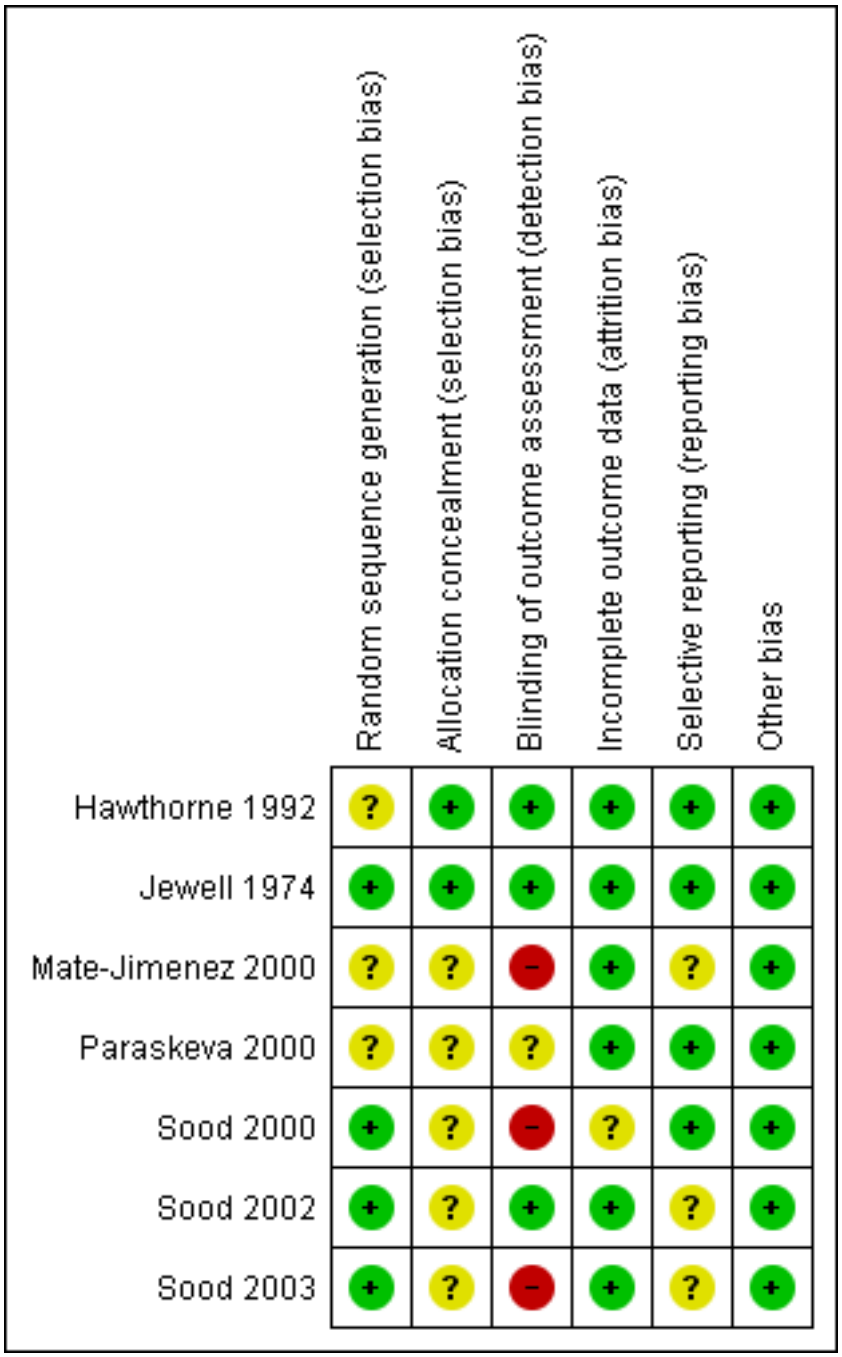
The time of follow up varied from 12 months to 76 weeks. All studies reported the proportion of patients relapsing, or remaining in remission. In addition there was information on reduction of clinical scores, safety data or number of relapses during the study period from single studies. There were no survival methods applied to evaluate the time to relapse.

The included studies are described in detail in the 'Characteristics of included studies' table" (Characteristics of included studies).

Risk of bias in included studies

The methodological quality of the studies was unsatisfactory in four of seven studies (See Figure 2). Four studies (Jewell 1974; Sood 2000; Sood 2002; Sood 2003), described adequate methods for sequence generation and were rated as low risk for this item. Three studies (Hawthorne 1992; Mate-Jimenez 2000; Paraskeva 2000) were rated as unclear risk because the methods used for randomization were not described. Appropriate methods for allocation concealment were reported or supplied by the authors for two of the seven studies (Hawthorne 1992; Jewell 1974), and these studies were rated as low risk of bias for this item. The other five studies (Mate-Jimenez 2000;Paraskeva 2000; Sood 2000; Sood 2002; Sood 2003), were rated as unclear for allocation concealment. Two of the three studies using active comparators were open label (Mate-Jimenez 2000; Sood 2003), and therefore at high risk of bias for lack of blinding. Paraskeva 2000 did not describe blinding in the abstract. The study by Sood 2000 was single blind (patients only) and was rated as high risk of bias for blinding. Six studies were rated as low risk for incomplete outcome data (Hawthorne 1992; Jewell 1974; Mate-Jimenez 2000; Paraskeva 2000; Sood 2002; Sood 2003). Three of the studies were rated at an unclear risk of bias for selective reporting because of missing outcomes (Sood 2003), or potential *post hoc* outcomes (Mate-Jimenez 2000; Sood 2002). The other four studies were rated as low risk for this item. Furthermore, it is of note that all studies were small (maximum number of patients: 80 (Jewell 1974)).

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Effects of interventions

See: **Summary of findings for the main comparison** Azathioprine versus placebo for maintenance of remission in ulcerative colitis; **Summary of findings 2** Azathioprine versus sulfasalazine for maintenance of remission in ulcerative colitis; **Summary of findings 3** 6-Mercaptopurine versus 5-ASA for maintenance of remission in ulcerative colitis; **Summary of findings 4** 6-Mercaptopurine versus methotrexate for maintenance of remission in ulcerative colitis; **Summary of findings 5** Azathioprine (AZA) versus cyclosporin of remission in ulcerative colitis; **Summary of findings 6** Adverse events across placebo-controlled and aminosalicylate-comparator trials

Azathioprine versus Placebo

Four studies (Hawthorne 1992; Jewell 1974; Sood 2000; Sood 2002), including 232 patients, compared azathioprine to placebo. A pooled analysis showed azathioprine was significantly superior to placebo for maintenance of remission. Forty-four per cent (51/115) of patients in the azathioprine group failed to maintain remission compared to 65% (76/117) of patients receiving placebo (RR 0.68, 95% CI 0.54 to 0.86). No heterogeneity was detected for this comparison (P = 0.60; I^2 = 0%). A GRADE analysis showed

that the overall quality of the evidence supporting this outcome was low due to a high risk of bias in one study in the pooled analysis (Sood 2000), and imprecision due to sparse data (127 events; See Summary of findings for the main comparison). The results were similar when analyses were restricted to patients after successful induction of remission; however, data on primary treatment failures were only available for two studies. Forty-six percent of patients in the azathioprine group failed to maintain remission compared to 67% of placebo patients (2 studies, 123 patients; RR 0.67, 95% CI 0.49 to 0.93). There was no obvious evidence for an effect by dose of azathioprine or use of co-medication in these studies, but the treatment schedules were too varied for formal testing. Also, a difference between azathioprine and 6-mercaptopurine could not be examined, as the only 6-mercaptopurine study was open label and did not use a placebo control (Mate-Jimenez 2000).

The four placebo-controlled studies also reported adverse event rates (Hawthorne 1992; Jewell 1974; Sood 2000; Sood 2002). Overall, azathioprine and placebo were not significantly different with regard to the risk of adverse events. Eight percent (9/115) of azathioprine patients and 3% (3/117) of placebo patients experienced at least one adverse event (232 patients, RR 2.51, 95% CI 0.82 to 7.74). No heterogeneity was detected for this comparison ($P = 0.48$; $I^2 = 0\%$). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to risk of bias and serious imprecision due to sparse data (12 events; See Summary of findings for the main comparison). Withdrawals due to adverse events were reported in three studies (Hawthorne 1992; Sood 2000; Sood 2002). A pooled analysis revealed no statistically significant difference in withdrawals due to adverse events. Four percent (3/75) of azathioprine patients withdrew due to adverse events compared to and 0% (0/77) of placebo patients (152 patients, RR 7.00, 95% CI 0.38 to 128.87). However, this result should be interpreted with caution due to the small sample size and a GRADE rating of very low (risk of bias, very sparse data and very wide confidence intervals; See Summary of findings for the main comparison).

Azathioprine or 6-Mercaptopurine versus 5-Aminosalicylate or Sulfasalazine

Sood 2003 compared azathioprine to sulfasalazine and Mate-Jimenez 2000 compared 6-mercaptopurine to 5-ASA therapy. A pooled analysis of these studies to assess failure to maintain remission was not possible due to a high degree of heterogeneity ($P = 0.03$; $I^2 = 79\%$). Sood 2003 reported that 58% (7/12) of azathioprine patients failed to maintain remission compared to 38% (5/13) of sulfasalazine patients (25 patients; RR 1.52, 95% CI 0.66 to 3.50). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to a high risk of bias (open label study), and serious imprecision due to sparse data (12 events; See Summary of findings 2). Adverse events occurred in 17% (2/12) and 0% (0/13) patients administered azathioprine and sulfasalazine, respectively (RR 5.38, 95% CI 0.28 to 101.96). All of the patients who experienced adverse events withdrew. Mate-Jimenez 2000 reported that 50% (7/14) of 6-mercaptopurine patients failed to maintain remission compared to 100% (8/8) of 5-aminosalicylate patients (22 patients; RR 0.53, 95% CI 0.31 to 0.90). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to a high risk of bias (open label study), and serious imprecision due to sparse data (15 events; See Summary of findings 3). Withdrawals due to adverse events by week 30 occurred in 21% (3/14) patients receiving 6-mercaptopurine compared to 0% (0/8) of patients receiving 5-aminosalicylates (RR 4.20, 95% CI 0.24 to 72.29). These results should be interpreted with caution as both studies were unblinded and had small sample sizes (Mate-Jimenez 2000; Sood 2003).

6-Mercaptopurine versus Methotrexate

Mate-Jimenez 2000 compared 6-mercaptopurine (n = 14) to methotrexate (n = 12). Fifty percent (7/14) of 6-mercaptopurine patients failed to maintain remission compared to 92% (11/12) of methotrexate patients (RR 0.55, 95% CI 0.31 to 0.95). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to a high risk of bias (open label study), and serious imprecision due to sparse data (18 events; See Summary of findings 4). Twenty-one percent (3/14) and 17% (2/12) patients receiving 6-mercaptopurine and methotrexate, respectively, withdrew due to adverse events (RR 1.29, 95% CI 0.26 to 6.46). These results should be interpreted with caution as the study was open label and had a small sample size.

Azathioprine vs Cyclosporin

Paraskeva 2000 evaluated the effectiveness of oral azathioprine (2.5 mg/kg/day) (n = 8) against oral cyclosporin (4 mg/kg/day) (n = 8) at maintaining remission after 6 months of therapy, coupled with tapering steroids and 5-ASA in patients with remissive UC, who had previously responded favourably to intravenous cyclosporin. The patients were followed for an additional 12 months to monitor for relapse after all medications were discontinued. At 18 moths, 50% (4/8) of patients receiving azathioprine had failed to maintain remission, compared to (62.5% (5/8) of those receiving cyclosporin (RR 0.80, 95% CI 0.33 to 1.92). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to unclear risk of bias (abstract publication), and serious imprecision due to sparse data (9 events; See Summary of findings 5). One patient from each group withdrew due to adverse events (RR 1.00 95% CI 0.07 to 13.37), and 4/4 of those completing the 6 months of treatment in the cyclosporin group had experienced at least 1 adverse event (RR 0.20 95% CI 0.03 to 1.35). These results should be interpreted with caution due to the small sample size and a GRADE rating of very low.

Adverse Events Across Placebo-controlled and Aminosalicylate-comparator Trials

We decided to pool comparators with a known low adverse effect profile (i.e. aminosalicylates) with the placebo-controlled studies to assess adverse effects. There was no statistically significant difference in the incidence of adverse events when these studies were pooled (Hawthorne 1992; Jewell 1974; Sood 2000; Sood 2002; Sood 2003). Nine per cent of azathioprine patients (11/127) experienced at least one adverse event compared to 2% (3/130) of control patients (RR 2.82, 95% CI 0.99 to 8.01). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to high risk of bias (blinding), and serious imprecision due to sparse data (14 events; See Summary of findings 6). When studies that reported withdrawal due to adverse events were pooled, there was a statistically significant difference in withdrawals due to adverse events. Eight per cent of patients in the azathioprine group (8/101) withdrew due to adverse events compared to 0% of control patients (RR 5.43, 95% CI 1.02 to 28.75). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to high risk of bias

(blinding), and serious imprecision due to sparse data (8 events; See Summary of findings 6). Pancreatitis was reported in 3 of 141 patients receiving antimetabolites. Jaundice or hepatitis was reported in 1 of 127 patients receiving azathioprine. Bone marrow suppression was reported for 5 of 127 patients on azathioprine. One case of pancreatitis was reported in a cyclosporin patient. Deaths, opportunistic infections or neoplasia were not reported. Mate-Jimenez 2000 reported several cases of bone marrow suppression (3 of 30 IBD patients receiving 6-mercaptopurine) in patients with IBD, some of whom had ulcerative colitis. Mate-Jimenez 2000 did not report separate the events by disease entity. Jewell 1974 did not formally withdraw patients with serious adverse events from the study, but paused therapy and restarted open label treatment. When studies that reported on acute pancreatitis were pooled, there was no statistically significant difference in the proportion of patients who developed pancreatitis. Two per cent of patients in the azathioprine/6-mercaptopurine group (3/141) developed pancreatitis compared to 0% of control patients (RR 4.13, 95% CI 0.48 to 35.48). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to high risk of bias (blinding), and serious imprecision due to sparse data (3 events; See Summary of findings 6). When studies that reported on bone marrow suppression were pooled, there was no statistically significant difference in the proportion of patients who developed bone marrow suppression. Four per cent of patients in the azathioprine group (5/127) developed bone marrow suppression compared to 0.8% (1/130) of control patients (RR 3.09, 95% CI 0.64 to 14.83). A GRADE analysis showed that the overall quality of the evidence supporting this outcome was very low due to high risk of bias (blinding), and serious imprecision due to sparse data (6 events; See Summary of findings 6).

DISCUSSION

Based on four trials (Hawthorne 1992; Jewell 1974; Sood 2000; Sood 2002), azathioprine was shown to be superior to placebo for the prevention of relapse in ulcerative colitis. The difference was statistically significant and appears to be clinically relevant. Relapse rates in the placebo groups ranged from 55% to 78%. Based on a mean failure rate of 65% in the combined placebo group, the pooled risk ratio of 0.68 would translate into a number needed to treat of 5 (absolute risk reduction 21%).

Several observations limit the reliability and clinical usefulness of this conclusion. Foremost, the quality of the trials was unsatisfactory for the majority of evaluated trials. Inadequate concealment of allocation has been shown to impact on effect sizes in clinical trials (Schulz 1995). Adequateness of concealment could not be assessed for five out of seven studies due to insufficient information (Mate-Jimenez 2000; Paraskeva 2000; Sood 2000; Sood 2002; Sood 2003). All of the trials had small sample sizes. Information on the assessment of relapse or remission remained mostly obscure, although most reported Baron's criteria for inclusion. The number of eligible trials was too small to assess the occurrence of publication bias, or any bias arising from differences in methodological quality.

In addition to these threats to the internal validity of the results, several issues arose concerning the applicability of the results to clinical practice. There were no long term studies. Moreover, it is unclear from the results of the included studies when it is appropriate to use azathioprine in clinical practice. Clinicians can prescribe purine antimetabolites when 5-ASA agents fail, or they can be used in addition to 5-ASA agents or instead of 5-ASA agents. The role of azathioprine as maintenance therapy in ulcerative colitis in the era of biologic agents is uncertain. These issues should be addressed in future studies.

Due to the limited number of trials and variable dosing of azathioprine we were unable to assess a dose-response relationship, or differences by use of co-medication or duration of treatment. Only two trials compared antimetabolites to current standard maintenance therapy with aminosalicylates (Mate-Jimenez 2000; Sood 2003). Both were of insufficient quality due to lack of blinding. One of these open label trials was the only study available that evaluated 6-mercaptopurine (Mate-Jimenez 2000).

For four of the included studies (Jewell 1974; Sood 2000; Sood 2002; Sood 2003), patients were randomized while in active disease. Applying an intention-to-treat analysis approach, we decided to include all patients randomized into the main analysis, including those who failed to reach remission. Strictly speaking, these trials were not mere maintenance trials, even though the analysis excluding induction failures rendered very similar results. There was no difference in the pooled results if the single withdrawal study was excluded (Hawthorne 1992), but this may be due to the small sample size. None of the trials included patient related outcomes such as quality of life or hospitalizations.

Case numbers were too small to conclusively assess the frequency of adverse events. However, the incidence of major adverse events in the treatment group was in accordance with previous reports from the literature, including a 2% risk of pancreatitis, and 4% risk of bone marrow suppression.

Azathioprine is commonly used as a maintenance treatment for patients with ulcerative colitis, however the lack of high quality trials evaluated in this review is remarkable. Considering the well established efficacy and safety of aminosalicylates for the maintenance of remission in ulcerative colitis (Marshall 2012; Wang 2016), antimetabolites cannot be recommended for first line treatment for this purpose. There may be a place for purine antimetabolites in patients who do not tolerate aminosalicylates. Moreover, purine antimetabolites may be appropriate maintenance treatment for patients who fail aminosalicylates or require induction with steroid therapy. Future high quality trials should allow for separate analysis of these patients, or use active comparators.

AUTHORS' CONCLUSIONS

Implications for practice

Azathioprine may be an effective treatment for maintaining remission in ulcerative colitis. It may be most useful in patients who have failed or cannot tolerate aminosalicylates, as well as patients who require steroid therapy to induce remission. However, there is insufficient evidence to assess superiority of azathioprine alone, or azathioprine in addition to standard maintenance, as compared to maintenance with aminosalicylates, methotrexate or cyclosporin. The effectiveness of azathioprine compared to biologic therapy is unknown. Overall, given the potential for serious adverse events, azathioprine may not be an ideal first line therapy in quiescent ulcerative colitis.

Implications for research

More data from good quality clinical trials are needed to assess the efficacy and safety of azathioprine for the maintenance of remission in ulcerative colitis as compared to standard maintenance therapy or biologic therapy. The question of when it is appropriate to use purine antimetabolite therapy in clinical practice should be addressed in future studies.

ACKNOWLEDGEMENTS

This work was based on a previous protocol by Steinhart AH, Hawkey CJ, and Modigliani R, first published in Issue 2, 1996.

Funding for the IBD/FBD Review Group (September 1, 2010 - August 31, 2015) has been provided by the Canadian Institutes of Health Research (CIHR) Knowledge Translation Branch (CON - 105145) and the CIHR Institutes of Nutrition, Metabolism and Diabetes (INMD); and Infection and Immunity (III) and the Ontario Ministry of Health and Long Term Care (HLTC3968FL-2010-2235).

Miss Ila Stewart has provided support for the IBD/FBD Review Group through the Olive Stewart Fund.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Hawthorne 1992

Methods	Randomized controlled trial

Hawthorne 1992 *(Continued)*

	Double blind, placebo controlled Withdrawal study Sudy duration 1 year
Participants	Diagnosis of ulcerative colitis In remission (or chronic low grade activity or steroid dependent) On azathioprine for 6 months or longer Only patients in remission used for review: n = 67
Interventions	Azathioprine continued at variable dosing (median 100 mg/day), P.O. Compared to azathioprine withdrawal - change to placebo Co-medication - continued from pre-trial medication: Sulfasalazine 1-4 g/day in 39 patients (mean 2 g/day) Mesalazine 0.8 to 3.2 g/day in 28 patients (mean 1.2g/day) None in 12 patients
Outcomes	Main: (time to) clinical relapse, one year relapse rate Secondary: adverse events
Notes	Additional information given by investigator, method of sequence generation was not remembered

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not explicitly discussed
Allocation concealment (selection bias)	Low risk	Centralised pharmacy-controlled randomisation Quote: "Randomisation was performed in hospital pharmacies"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "double blind" Quote: "an equivalent number of placebo tablets of identical appearance"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All withdrawals were noted and explained Intention-to-treat was followed
Selective reporting (reporting bias)	Low risk	All outcomes were reported
Other bias	Low risk	Nothing of note

Jewell 1974

Methods	Randomized controlled trial Double blind, placebo controlled Induction period included (steroid tapering) Study duration 1 year
Participants	Diagnosis of ulcerative colitis Active disease n = 80

Jewell 1974 *(Continued)*

Interventions	Azathioprine 2.5 mg/kg/d for 3 months, then dose reduction to 1.5 to 2.0 mg/kg/d, P.O. Compared to placebo Co-medication induction period: Outpatients/mild attack: prednisolone 20 mg/day P.O. and prednisolone enema for 1 months, then tail off over 2 weeks Inpatient/severe attack: nil per mouth, i.v. steroids 40 mg, tetracycline, topical steroids, for 4 days, then prednisolone 40 mg P.O., taper off Co-medication in maintenance: none
Outcomes	Main: Number of relapses during 1 year Maintenance of remission over 1 year Secondary: Failure to achieve remission Clinical severity of attacks, endoscopic and histological grading Adverse events Immunological changes
Notes	Additional information on randomization/methods supplied by the author

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Author correspondence
Allocation concealment (selection bias)	Low risk	Centralised randomisation Quote: "trial treatment was prescribed as 'azathioprine special' and the hospital pharmacists worked from a master sheet indicating whether a particular patient was to be given real or dummy azathioprine"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Double-blind, double-dummy Quote: "dummy azathioprine" Quote: "trial treatment was prescribed as 'azathioprine special' and the hospital pharmacists worked from a master sheet indicating whether a particular patient was to be given real or dummy azathioprine"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All 80 patients completed the trial
Selective reporting (reporting bias)	Low risk	All outcomes were reported
Other bias	Low risk	Nothing of note

Mate-Jimenez 2000

Methods	Randomized controlled trial open label, active controls (3 treatment arms) Study duration: 30 weeks induction period, 76 weeks maintenance (numbers also available for 48 to 56 weeks)
Participants	Diagnosis of ulcerative colitis (and Crohn's disease)

Mate-Jimenez 2000 *(Continued)*

	Outpatients, steroid dependent (steroids > 20 mg or frequent relapses) Maintenance part: successful withdrawal of steroids Only ulcerative colitis used for review: n = 34 (20 in maintenance)
Interventions	6-mp 1.5 mg/kg/day; decreased to 1 mg/kg/day once in remission a) compared to methotrexate 15 mg/week; decreased to 10 mg once in remission b) compared to 5-ASA 3 g/day Co-medication induction phase: Prednisolone continued from pre-trial dosis, max. 1 mg/kg/day for 2 more weeks Followed by tapering by 8 mg/week, depending on clinical status Co-medication maintenance phase: Antidiarrhoeals and folic acid as needed
Outcomes	Main: attaining remission (induction phase) Maintaining remission (maintenance phase) Secondary: adverse events, compliance
Notes	Information received from author not sufficient to assess randomization /allocation procedure

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not explicitly discussed
Allocation concealment (selection bias)	Unclear risk	Not described in published study
Blinding of outcome assessment (detection bias) All outcomes	High risk	Not explicitly discussed in the publish study, it was assumed to be open label
Incomplete outcome data (attrition bias) All outcomes	Low risk	24/72 patients withdrew in the first 30 weeks of the trial with reasons described. Worst outcome assumed in analysis
Selective reporting (reporting bias)	Unclear risk	All outcomes were reported, as well as some *post hoc* comparisons
Other bias	Low risk	No other issues

Paraskeva 2000

Methods	Randomized, active control trial
Participants	Patients (N = 16), aged 18-58 years, with acute, steroid resistant Ulcerative colitis, which had previously responded and tolerated IV cyclosporin (4 mg/kg/day). Pateints were in maintenance therapy on tapered steroids and 5-ASA
Interventions	Group A: n = 8, oral azathioprine (2.5 mg/kg/day) for 6 months Group B: n = 8, oral cyclosporin (4 mg/kg/day) for 6 months

Paraskeva 2000 *(Continued)*

	All patients were on tapering steroids and were on maintenance therapy with 5-ASA Follow-up after medication discontinuation for another 12 months
Outcomes	Primary outcome: Proportion of patients still in remission Secondary outcome: Safety data
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described in abstract
Allocation concealment (selection bias)	Unclear risk	Not described in abstract
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not described in abstract
Incomplete outcome data (attrition bias) All outcomes	Low risk	Missing/withdrawn data was accounted for with explanation. Intention to treat analysis used
Selective reporting (reporting bias)	Low risk	No protocol was available, all outcomes were reported
Other bias	Low risk	The study appears not to have any other forms of bias

Sood 2000

Methods	Randomized controlled trial Single (patient) blind placebo controlled Induction period included (steroid tapering) Study duration 12 months
Participants	Diagnosis of ulcerative colitis Relapse within 2 months of steroid withdrawal following successful induction of remission n = 50
Interventions	Azathioprine 2.0 mg/kg/d for 1 year Compared to placebo Co-medication induction period: i.v. hydrocortisone 400 mg/day, sulfasalazine 6-8 g/day, ciprofloxacin and metronidazole for 5 days Taper off of steroids to 1 mg/kg/day P.O., then by 10 mg every 10 days to 20 mg, then by 5 mg every 10 days Co-medication maintenance: sulfasalazine 6 g/day
Outcomes	Main: Complete or partial remission at 1 year (after successful induction) Secondary: adverse events

Sood 2000 *(Continued)*

Notes	The author was approached for clarification of the randomization and allocation concealment; no response was received

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "pseudorandom numbers ranging from 0-1 generated by a scientific calculator"
Allocation concealment (selection bias)	Unclear risk	Not described in the published study
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "single-blind" Quote: "identical matched placebo drugs" Quote: "drugs were provided by a single coordinator (VK) to the patients in identical blister packs" Quote: "The treating physician was aware of the drug treatment"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Quote: "Five patients...were excluded because of noncompliance and violation of treatment protocol" It is difficult to determine how these were dealt with in the analysis
Selective reporting (reporting bias)	Low risk	All outcome were reported
Other bias	Low risk	Nothing of note

Sood 2002

Methods	Randomized controlled trial Double blind placebo controlled Induction period included (steroid tapering) Study duration 12 months
Participants	Newly diagnosed ulcerative colitis, severely active (SEO > 220) n = 35
Interventions	Azathioprine 2.5 mg/kg/d for 1 year Compared to placebo Co-medication induction period: i.v. hydrocortisone 400 mg/day, sulfasalazine 6-8 g/day, ciprofloxacin and metronidazole for 7 days Taper off of steroids over 12-16 weeks
Outcomes	Main: Maintenance of remission (after successful induction) Number of patients suffering clinical relapse within 1 year Secondary: Course of mean activity index over 1 year Induction period: time to remission Adverse events

Sood 2002 *(Continued)*

Notes	The author was approached for clarification of the randomization and allocation concealment; no response was received

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "pseudo-random numbers ranging from 0-1, generated by a scientific calculator"
Allocation concealment (selection bias)	Unclear risk	Not described in published study
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "double blind" Quote: "identical matched placebo drugs" Quote: "The drugs were provided by a single coordinator (V.K.) to the patients in identical blister packs"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "All the 35 patients completed the study period"
Selective reporting (reporting bias)	Unclear risk	All data was reported, but also hemoglobin and albumin levels which appear to post hoc
Other bias	Low risk	Nothing of note

Sood 2003

Methods	Randomized controlled trial open label, active comparison (sulfasalazine) Induction period included (steroid tapering) Study duration 18 months
Participants	Newly diagnosed ulcerative colitis, severely active (SEO > 220) n = 25
Interventions	Azathioprine 2.5 mg/kg/day for 18 months Compared to sulfasalazine 6 g/day Co-medication induction period: prednisolone 1 mg/kg/day; Taper off by 10 mg/day every fortnight until 20 mg/day, then 5 mg/day every 2 weeks Co-medication maintenance: not reported
Outcomes	Main: maintenance of remission (after successful induction) for 18 months Treatment failure within 18 months Secondary: Failure to attain remission in induction phase Course of mean activity index Adverse events
Notes	The author was approached for clarification of the randomization and allocation concealment; no response was received

Risk of bias

Sood 2003 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "generating pseudo-random numbers ranging from 0-1 using a scientific calculator"
Allocation concealment (selection bias)	Unclear risk	Unclear, not described in the published study
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "open-label"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Two withdrawals due to side effects
Selective reporting (reporting bias)	Unclear risk	All outcomes reported, except endoscopic evaluation
Other bias	Low risk	Nothing of note

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

Study	Reason for exclusion
Ardizzone 1997	Retrospective study of patients with either steroid resistant or steroid dependant ulcerative colitis receiving azathioprine
Ardizzone 2006	This was a 6 month trial of patients with active steroid dependent ulcerative colitis. They were randomized to receive azathioprine or 5-aminosalicylic acid
Cassinotti 2009	Retrospective study of patients who discontinued azathioprine while in steroid free remission
Chebli 2010	Prospective non-randomized study of patients receiving azathioprine for steroid dependent ulcerative colitis
Cuffari 2004	Prospective non-randomized study of patients on azathioprine or 6-mercaptopurine
Domenech 2002	Retrospective chart review.
Fernandez-Banares 1996	Case series, not an RCT.
Hibi 2003	Two different studies, one a retrospective study of 82 inflammatory bowel disease patients receiving azathioprine or 6-mercaptopurine to assess the frequency of TMPT gene mutation. The other, a prospective non-randomized trial of 22 patients receiving azathioprine
Holtmann 2006	Retrospective chart review of 1176 patients with inflammatory bowel disease to evaluate the effect of azathioprine therapy
Kirk 1982	Patients had chronic active ulcerative colitis
Mantzaris 2004	All patients received azathioprine
Paoluzi 2002	Not an RCT, no control group

Study	Reason for exclusion
Rosenberg 1975	This was a randomized double-blind trial involving patients with active ulcerative colitis. Patients received placebo or azathioprine (1.5 mg/kg/day)
Sakuraba 2012	This was an open label pilot study examining granulocyte and monocyte apheresis against mercaptopurine therapy. Apheresis was not considered standard maintenance therapy

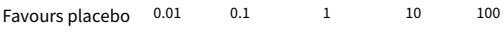
DATA AND ANALYSES

Comparison 1. Azathioprine versus placebo

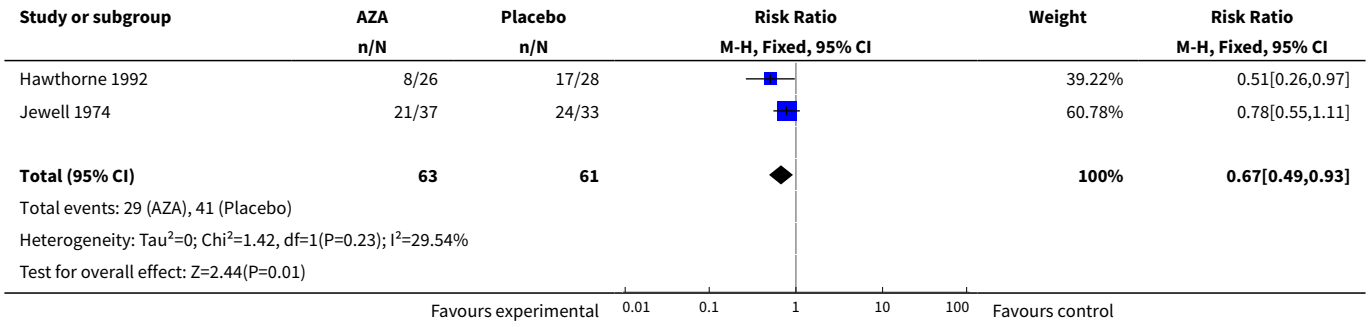
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Failure to maintain remission	4	232	Risk Ratio (M-H, Fixed, 95% CI)	0.68 [0.54, 0.86]
2 Failure to maintain remission in successfully induced cases only	2	124	Risk Ratio (M-H, Fixed, 95% CI)	0.67 [0.49, 0.93]
3 Any adverse event	4	232	Risk Ratio (M-H, Fixed, 95% CI)	2.51 [0.82, 7.74]
4 Withdrawal due to adverse event	3	152	Risk Ratio (M-H, Fixed, 95% CI)	7.0 [0.38, 128.87]

Analysis 1.1. Comparison 1 Azathioprine versus placebo, Outcome 1 Failure to maintain remission.

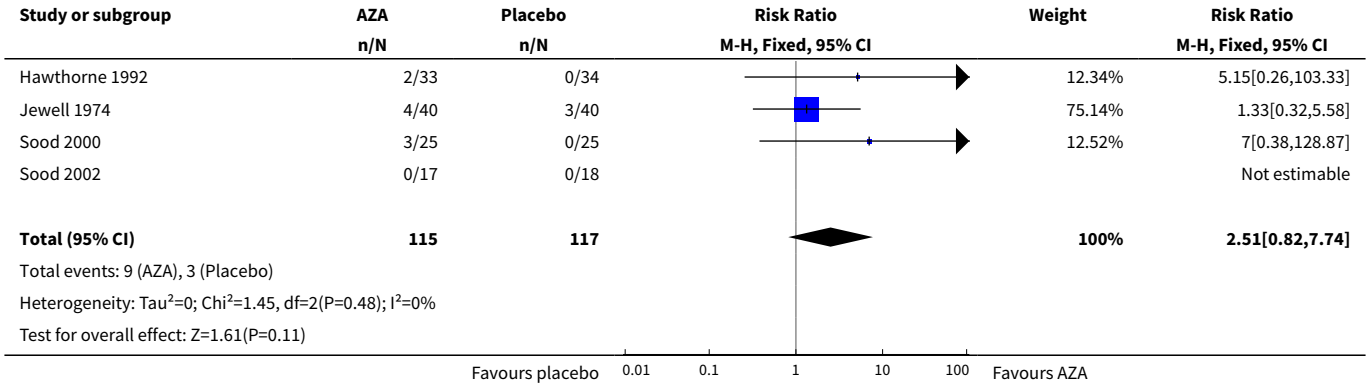
Study or subgroup	AZA n/N	Placebo n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Hawthorne 1992	12/33	20/34		26.12%	0.62[0.36,1.05]
Jewell 1974	24/40	31/40		41.11%	0.77[0.57,1.05]
Sood 2000	11/25	15/25		19.89%	0.73[0.42,1.27]
Sood 2002	4/17	10/18		12.88%	0.42[0.16,1.1]
Total (95% CI)	**115**	**117**		**100%**	**0.68[0.54,0.86]**
Total events: 51 (AZA), 76 (Placebo)					
Heterogeneity: Tau^2=0; Chi^2=1.85, df=3(P=0.6); I^2=0%					
Test for overall effect: Z=3.16(P=0)					



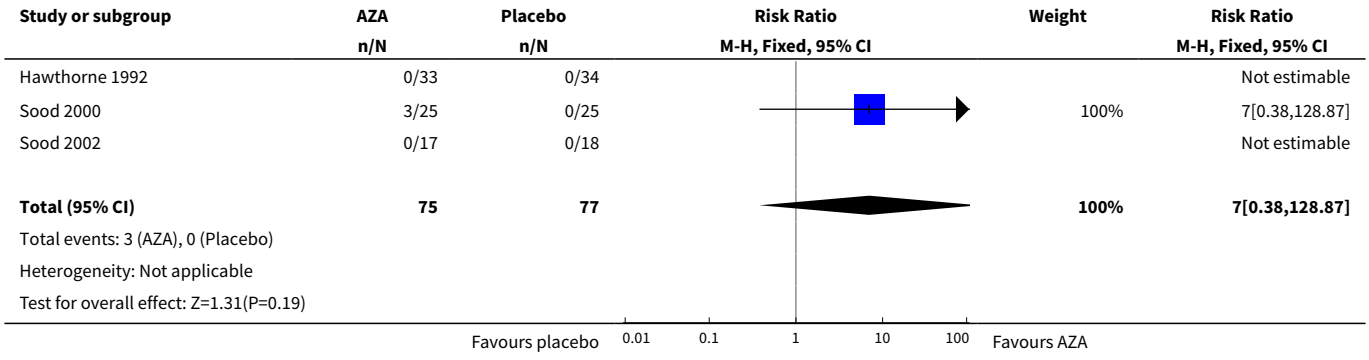
Analysis 1.2. Comparison 1 Azathioprine versus placebo, Outcome 2 Failure to maintain remission in successfully induced cases only.



Analysis 1.3. Comparison 1 Azathioprine versus placebo, Outcome 3 Any adverse event.



Analysis 1.4. Comparison 1 Azathioprine versus placebo, Outcome 4 Withdrawal due to adverse event.

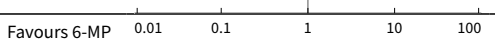


Comparison 2. Azathioprine or 6-mercaptopurine versus 5-ASA or sulfasalzine

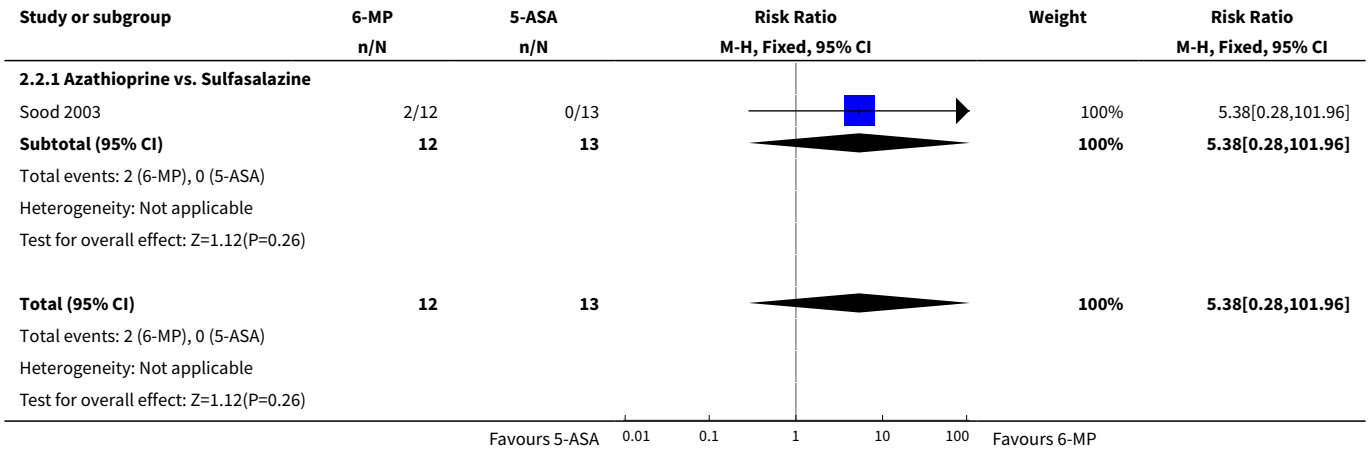
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Failure to maintain remission	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 6-Mercaptopurine vs. 5-ASA	1	22	Risk Ratio (M-H, Fixed, 95% CI)	0.53 [0.31, 0.90]
1.2 Azathioprine vs. Sulfasalazine	1	25	Risk Ratio (M-H, Fixed, 95% CI)	1.52 [0.66, 3.50]
2 Any adverse event	1	25	Risk Ratio (M-H, Fixed, 95% CI)	5.38 [0.28, 101.96]
2.1 Azathioprine vs. Sulfasalazine	1	25	Risk Ratio (M-H, Fixed, 95% CI)	5.38 [0.28, 101.96]
3 Withdrawal due to adverse event	2	47	Risk Ratio (M-H, Fixed, 95% CI)	4.72 [0.61, 36.35]
3.1 6-Mercaptopurine vs. 5-ASA	1	22	Risk Ratio (M-H, Fixed, 95% CI)	4.2 [0.24, 72.29]
3.2 Azathioprine vs. Sulfasalazine	1	25	Risk Ratio (M-H, Fixed, 95% CI)	5.38 [0.28, 101.96]

Analysis 2.1. Comparison 2 Azathioprine or 6-mercaptopurine versus 5-ASA or sulfasalzine, Outcome 1 Failure to maintain remission.

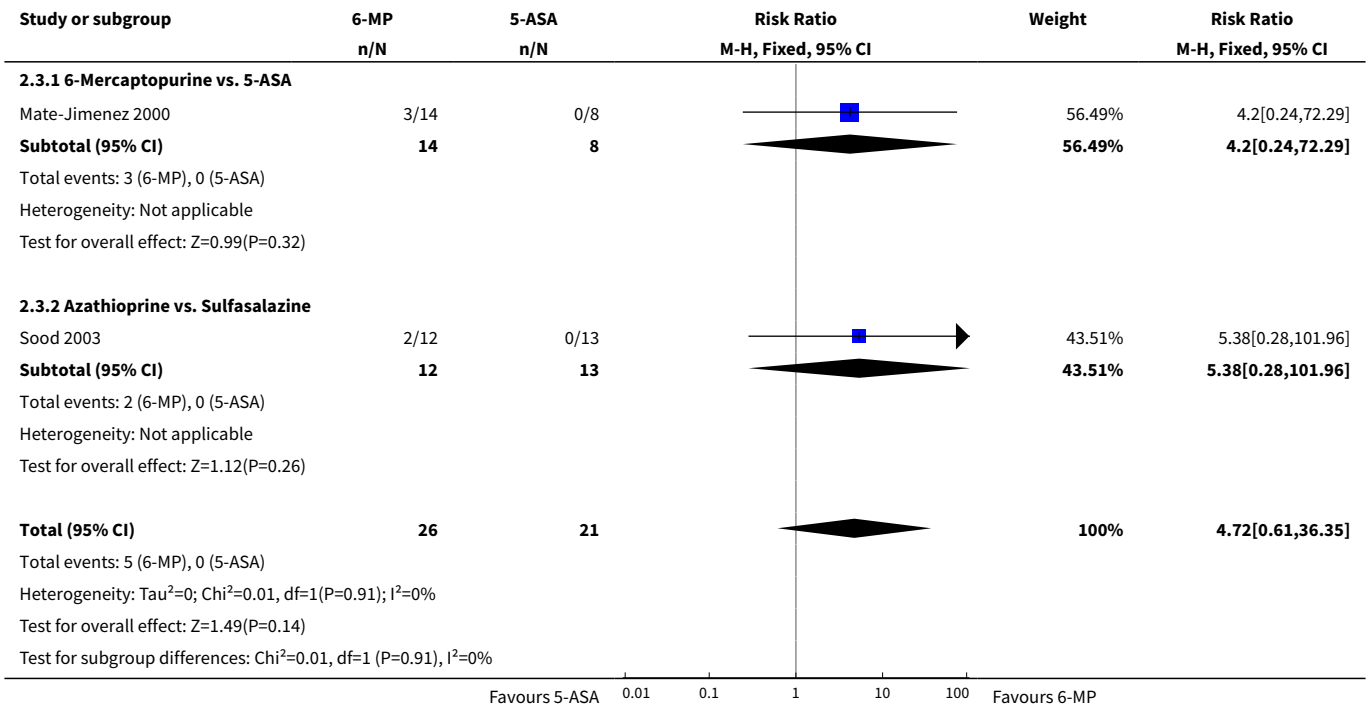
Study or subgroup	6-MP n/N	5-ASA n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
2.1.1 6-Mercaptopurine vs. 5-ASA					
Mate-Jimenez 2000	7/14	8/8		100%	0.53[0.31,0.9]
Subtotal (95% CI)	**14**	**8**		**100%**	**0.53[0.31,0.9]**
Total events: 7 (6-MP), 8 (5-ASA)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.35(P=0.02)					
2.1.2 Azathioprine vs. Sulfasalazine					
Sood 2003	7/12	5/13		100%	1.52[0.66,3.5]
Subtotal (95% CI)	**12**	**13**		**100%**	**1.52[0.66,3.5]**
Total events: 7 (6-MP), 5 (5-ASA)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.97(P=0.33)					
Test for subgroup differences: Chi²=4.33, df=1 (P=0.04), I²=76.91%					



Analysis 2.2. Comparison 2 Azathioprine or 6-mercaptopurine versus 5-ASA or sulfasalzine, Outcome 2 Any adverse event.



Analysis 2.3. Comparison 2 Azathioprine or 6-mercaptopurine versus 5-ASA or sulfasalzine, Outcome 3 Withdrawal due to adverse event.

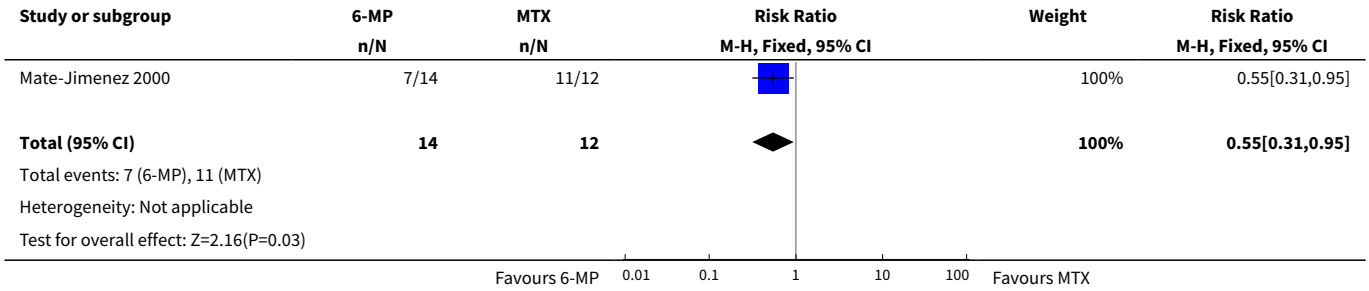


Comparison 3. 6-Mercaptopurine versus methotrexate

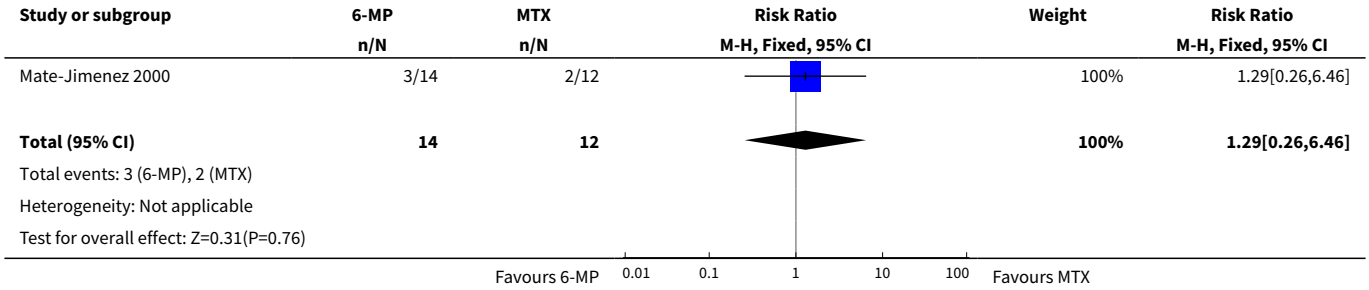
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Failure to maintain remission	1	26	Risk Ratio (M-H, Fixed, 95% CI)	0.55 [0.31, 0.95]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2 Withdrawal due to adverse event	1	26	Risk Ratio (M-H, Fixed, 95% CI)	1.29 [0.26, 6.46]

Analysis 3.1. Comparison 3 6-Mercaptopurine versus methotrexate, Outcome 1 Failure to maintain remission.



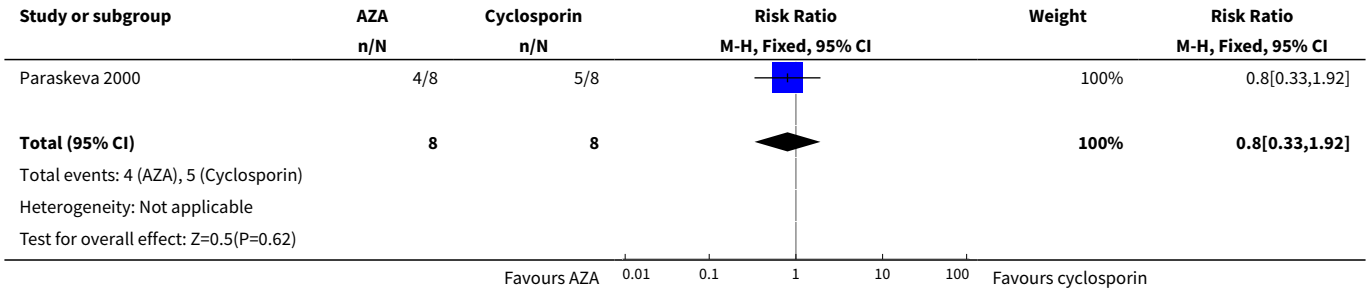
Analysis 3.2. Comparison 3 6-Mercaptopurine versus methotrexate, Outcome 2 Withdrawal due to adverse event.



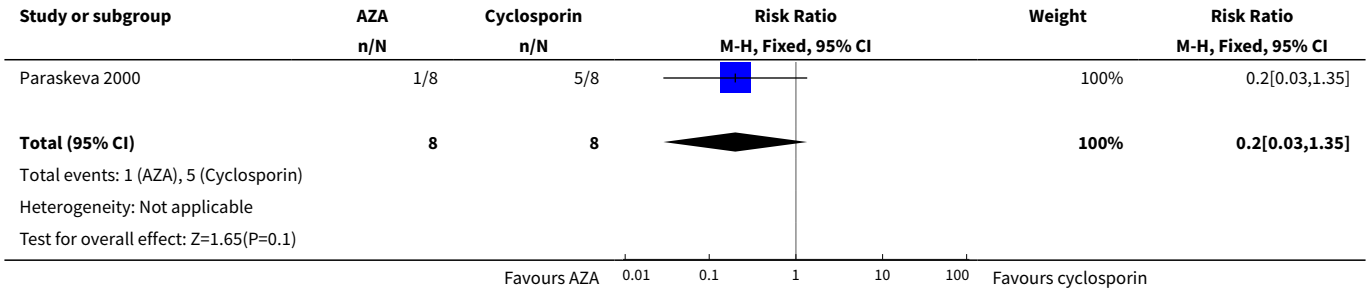
Comparison 4. Azathioprine versus cyclosporin

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Failure to maintain remission	1	16	Risk Ratio (M-H, Fixed, 95% CI)	0.8 [0.33, 1.92]
2 Any adverse event	1	16	Risk Ratio (M-H, Fixed, 95% CI)	0.2 [0.03, 1.35]
3 Withdrawal due to an adverse event	1	16	Risk Ratio (M-H, Fixed, 95% CI)	1.0 [0.07, 13.37]

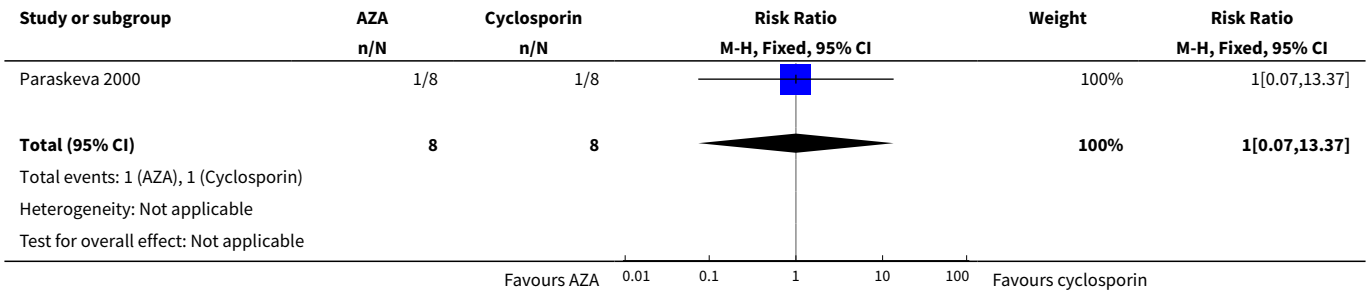
Analysis 4.1. Comparison 4 Azathioprine versus cyclosporin, Outcome 1 Failure to maintain remission.



Analysis 4.2. Comparison 4 Azathioprine versus cyclosporin, Outcome 2 Any adverse event.



Analysis 4.3. Comparison 4 Azathioprine versus cyclosporin, Outcome 3 Withdrawal due to an adverse event.



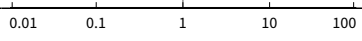
Comparison 5. Adverse events - all trials

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Any adverse event	5	257	Risk Ratio (M-H, Fixed, 95% CI)	2.82 [0.99, 8.01]
2 Withdrawal due to adverse event	5	199	Risk Ratio (M-H, Fixed, 95% CI)	5.43 [1.02, 28.75]
3 Acute pancreatitis	6	279	Risk Ratio (M-H, Fixed, 95% CI)	4.13 [0.48, 35.48]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4 Bone marrow suppression	5	257	Risk Ratio (M-H, Fixed, 95% CI)	3.09 [0.64, 14.83]

Analysis 5.1. Comparison 5 Adverse events - all trials, Outcome 1 Any adverse event.

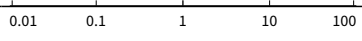
Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Hawthorne 1992	2/33	0/34		11.01%	5.15[0.26,103.33]
Jewell 1974	4/40	3/40		67.05%	1.33[0.32,5.58]
Sood 2000	3/25	0/25		11.18%	7[0.38,128.87]
Sood 2002	0/17	0/18			Not estimable
Sood 2003	2/12	0/13		10.76%	5.38[0.28,101.96]
Total (95% CI)	**127**	**130**		**100%**	**2.82[0.99,8.01]**
Total events: 11 (Treatment), 3 (Control)					
Heterogeneity: Tau^2=0; Chi^2=1.77, df=3(P=0.62); I^2=0%					
Test for overall effect: Z=1.95(P=0.05)					



Favours experimental — Favours control

Analysis 5.2. Comparison 5 Adverse events - all trials, Outcome 2 Withdrawal due to adverse event.

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Hawthorne 1992	0/33	0/34			Not estimable
Mate-Jimenez 2000	3/14	0/8		38.9%	4.2[0.24,72.29]
Sood 2000	3/25	0/25		31.12%	7[0.38,128.87]
Sood 2002	0/17	0/18			Not estimable
Sood 2003	2/12	0/13		29.97%	5.38[0.28,101.96]
Total (95% CI)	**101**	**98**		**100%**	**5.43[1.02,28.75]**
Total events: 8 (Treatment), 0 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.06, df=2(P=0.97); I^2=0%					
Test for overall effect: Z=1.99(P=0.05)					

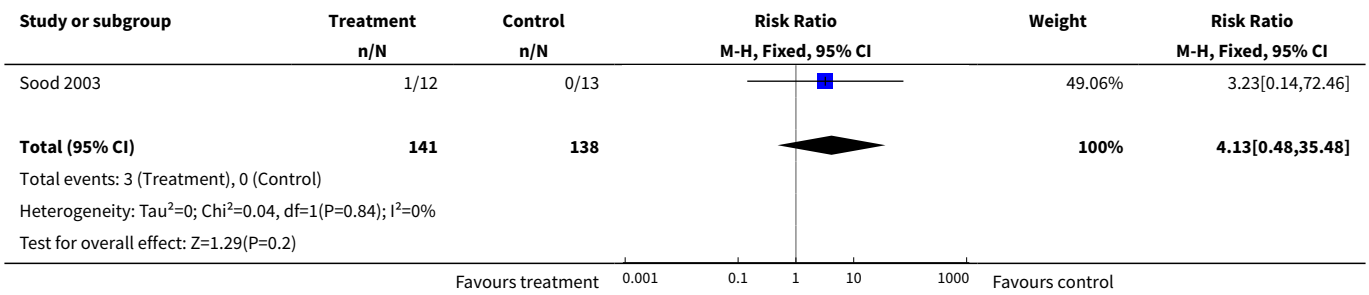


Favours experimental — Favours control

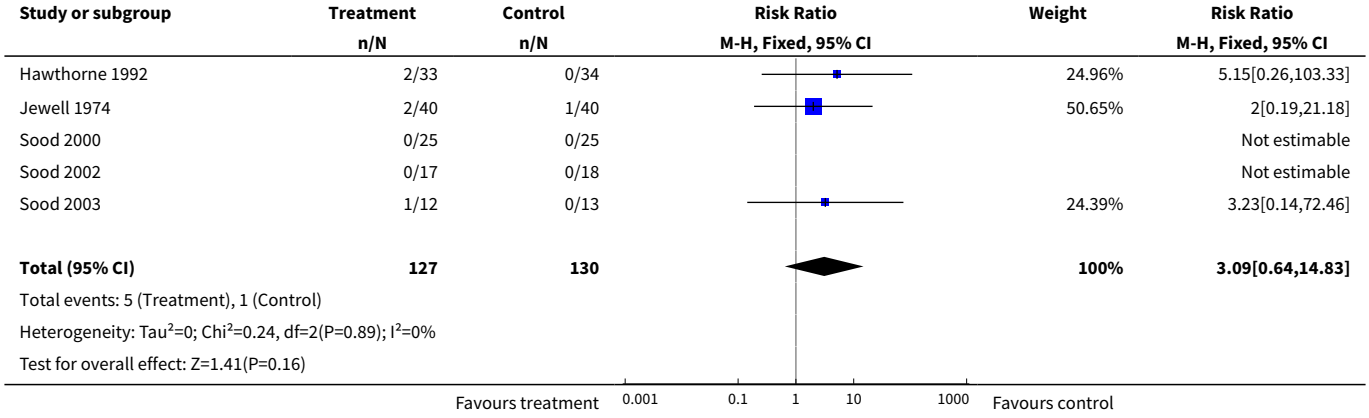
Analysis 5.3. Comparison 5 Adverse events - all trials, Outcome 3 Acute pancreatitis.

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Hawthorne 1992	0/33	0/34			Not estimable
Jewell 1974	0/40	0/40			Not estimable
Mate-Jimenez 2000	0/14	0/8			Not estimable
Sood 2000	2/25	0/25		50.94%	5[0.25,99.16]
Sood 2002	0/17	0/18			Not estimable

Favours treatment — Favours control



Analysis 5.4. Comparison 5 Adverse events - all trials, Outcome 4 Bone marrow suppression.



APPENDICES

Appendix 1. Electronic Search Strategy

EMBASE

1. random$.tw.

2. factorial$.tw.

3. (crossover$ or cross over$ or cross-over$).tw.

4. placebo$.tw.

5. single blind.mp.

6. double blind.mp.

7. triple blind.mp.

8. (singl$ adj blind$).tw.

9. (double$ adj blind$).tw.

10. (tripl$ adj blind$).tw.

11. assign$.tw.

12. allocat$.tw.

13. crossover procedure/

14. double blind procedure/

15. single blind procedure/

16. triple blind procedure/

17. randomized controlled trial/

18. or/1-17

19. (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)

20. 18 not 19

21. (anti-metabolite* or anti-metabolite* or antimetabolite*).mp.

22. (AZA or azathioprine).mp.

23. (6-mercaptopurine or mercaptopurine or 6-MP or 6MP).mp.

24. azathioprine.mp. or exp azathioprine/

25. ulcerative colitis.mp. or exp ulcerative colitis/

26. inflammatory bowel disease*.mp.

27. IBD.mp.

28. or/21-24

29. or/25-27

30. 20 and 28 and 29

Results: 696

MEDLINE

1. random$.tw.

2. factorial$.tw.

3. (crossover$ or cross over$ or cross-over$).tw.

4. placebo$.tw.

5. single blind.mp.

6. double blind.mp.

7. triple blind.mp.

8. (singl$ adj blind$).tw.

9. (double$ adj blind$).tw.

10. (tripl$ adj blind$).tw.

11. assign$.tw.

12. allocat$.tw.

13. crossover procedure/

14. double blind procedure/

15. single blind procedure/

16. triple blind procedure/

17. randomized controlled trial/

18. or/1-17

19. (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)

20. 18 not 19

21. (anti-metabolite* or anti-metabolite* or antimetabolite*).mp.

22. (AZA or azathioprine).mp.

23. (6-mercaptopurine or mercaptopurine or 6-MP or 6MP).mp.

24. azathioprine.mp. or exp azathioprine/

25. ulcerative colitis.mp. or exp ulcerative colitis/

26. inflammatory bowel disease*.mp.

27. IBD.mp.

28. or/21-24

29. or/25-27

30. 20 and 28 and 29

Results: 163

Cochrane Library search strategy:

#1 ulcerative colitis or UC
#2 inflammatory bowel disease or IBD
#3 MeSH descriptor Colitis, Ulcerative explode all trees
#4 MeSH descriptor Inflammatory Bowel Diseases explode all trees
#5 (#1 OR #2 OR #3 OR #4)
#6 anti-metabolite* or anti metabolite* or antimetabolite*
#7 MeSH descriptor Antimetabolites explode all trees
#8 6-mercaptopurine or mercaptopurine or 6-MP
#9 MeSH descriptor 6-Mercaptopurine explode all trees
#10 aza or azathioprine
#11 MeSH descriptor Azathioprine explode all trees
#12 (#6 OR #7 OR #8 OR #9 OR #10 OR #11)
#13 (#5 AND #12)

Results: 223

IBD Specialized Register

Title:(6-mercaptopurine or mercaptopurine or 6-MP or 6MP or aza or azathioprine) and (colitis or UC or inflammatory bowel disease or IBD)

Results: 0

Total: 1082

WHAT'S NEW

Date	Event	Description
30 July 2015	New citation required but conclusions have not changed	Updated review with new authors

Date	Event	Description
30 July 2015	New search has been performed	New literature searches conducted on 30 July 2015. One new included study added

DECLARATIONS OF INTEREST

Antje Timmer received grants (paid to institution) from Sanofi-Aventis, Bayer, Takeda, Celgene, and Novartis for pharmacoepidemiological studies prior to 2014; and payment for lectures from Abbott, Ferring, The Falk Foundation, and MSD Sharp. All of these activities are outside the submitted work.

Petrease H Patton: None known.

Nilesh Chande has received fees for consultancy from Abbott/AbbVie , Ferring, and Actavis; fees for lectures from Abbott, travel expenses from Merck and has stock/stock options in Pfizer, Glaxo Smith Kline, Proctor and Gamble and Johnson and Johnson. All of these financial activities are outside the submitted work.

John WD McDonald: None known.

John K MacDonald: None known.

INDEX TERMS

Medical Subject Headings (MeSH)

Anti-Inflammatory Agents, Non-Steroidal [adverse effects] [therapeutic use]; Antimetabolites [adverse effects] [*therapeutic use]; Azathioprine [adverse effects] [*therapeutic use]; Colitis, Ulcerative [*drug therapy]; Maintenance Chemotherapy [*methods]; Mercaptopurine [adverse effects] [*therapeutic use]; Mesalamine [therapeutic use]; Randomized Controlled Trials as Topic; Secondary Prevention; Sulfasalazine [therapeutic use]

MeSH check words

Adult; Humans