REVIEW

Thiopurines and inflammatory bowel disease: Current evidence and a historical perspective

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Abstract

The use of thiopurines in inflammatory bowel disease (IBD) has been examined in numerous prospective, controlled trials, with a majority demonstrating a clinical benefit. We conducted this review to describe the historical and current evidence in the use of thiopurines in IBD. A systematic search was performed on MEDLINE between 1965 and 2016 to identify studies on thiopurines in IBD. The most robust evidence for thiopurines in IBD includes induction of remission in combination with anti-tumor necrosis factor (anti-TNF) agents, and maintenance of remission and post-operative maintenance in Crohn's disease. Less evidence exists for thiopurine monotherapy in induction of remission, maintenance of ulcerative colitis, chemo-prevention of colorectal cancer, and in preventing immunogenicity to anti-TNF. Evidence was often limited by trial design. Overall, thiopurines have demonstrated efficacy in a broad range of presentations of IBD. With more efficacious novel therapeutic agents, the positioning of thiopurines in the management of IBD will change and future studies will analyze the benefit of thiopurines alone and in conjunction with these new medications.

Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Thiopurines; Mercaptopurine; Azathioprine

Core tip: In this review, we systematically describe the historical and current evidence in the use of thiopurines in inflammatory bowel disease (IBD). The most robust evidence for thiopurines in IBD includes induction of remission in combination with anti-tumor necrosis factor agents, and maintenance of remission and post-operative maintenance in Crohn's disease. With more effective and newer therapeutic agents, the positioning of thiopurines in the management of IBD

will change. Future studies should examine the benefit of thiopurines alone and in conjunction with these novel agents.

INTRODUCTION

Historically, the use of thiopurines, mercaptopurine and azathioprine, purine antagonists which inhibit DNA and RNA synthesis (Figure 1), in the treatment of inflammatory bowel disease (IBD) was based upon their efficacy in other autoimmune disorders, including systemic lupus erythematous and rheumatoid arthritis[1]. The efficacy of thiopurines in both Crohn's disease (CD) and ulcerative colitis (UC) has been documented in prospective, double-blind, placebo-controlled trials, with data supporting their beneficial therapeutic effects in inducing and maintaining disease remission, postoperative maintenance in CD, and chemoprevention of colorectal cancer (CRC)[2-6]. In addition, the medication has taken on an important role in conjunction with anti-tumor necrosis factor (TNF) therapy by interfering with antibody production[7].

Despite this evidence demonstrating the efficacy of thiopurine agents, there exists a hesitation with their clinical use. This may be based upon the fact that some of the trials were withdrawal designed[8], pediatric-based[9], recruited a small number of patients, or utilized a scoring system not universally accepted[10]. Furthermore, their role in infectious as well as malignant complications has been scrutinized.

In this review, undertaken after the passing of Dr. Daniel Present, we will review the historical basis, current evidence, and clinical experience in the use of thiopurines at various stages of IBD. We will also comment on how its use has changed over time and postulate on its positioning in the future. Lastly, this review will be accompanied by an experience overview by Dr. Daniel Present and Dr. Burton Korelitz, co-investigators on the seminal paper on the use of thiopurines in IBD[10].

For completeness, we conducted a systematic electronic search for relevant full-text articles in English using the MEDLINE database between January 1, 1965 and January 1, 2016. We used search terms associated with IBD and thiopurines, including "inflammatory bowel disease", "Crohn's disease", or "ulcerative colitis" in combination with "thiopurines", "azathioprine", "mercaptopurine", and "6-mercaptopurine". Reference lists from retrieved studies and review articles were examined to identify additional studies of relevance.

Preference was given to high impact articles with randomized trial designs.

THIOPURINES FOR INDUCTION OF REMISSION IN CD

A number of controlled clinical trials have investigated the efficacy of thiopurines in the treatment of active CD. The results of the four earliest trials were published in the 1970s[11-14]. The first three studies investigating azathioprine were small (enrolling 12-16 patients), utilized varying doses of drug (ranging from 2 to 4 mg/kg/d), and followed patients for a maximum of 24 wk. The response rates in these studies varied from 36%-100%. The largest of these initial trials was reported by Summers *et al*[13] in 1979, and involved a 17-wk randomized, double-blind, placebo-controlled trial of azathioprine 2.5 mg/kg/d in 136 patients with active CD (defined as a Crohn's Disease Activity Index (CDAI) score > 150). The rates of remission (CDAI < 150 at week 17) with azathioprine (36%; 21/59) were superior to placebo (26%; 20/77), although not at the level of statistical significance.

The first long-term study to demonstrate the efficacy of mercaptopurine to induce remission was reported by Present *et al*[10] in 1980. Eighty-three chronically ill patients with CD were entered into a two-year double-blind study comparing mercaptopurine 1.5 mg/kg with placebo. Crossover data showed that improvement occurred in 67% of courses of mercaptopurine compared with 8% of courses of placebo (*P* < 0.001). Mercaptopurine was also found to be more effective than placebo in fistula closure and steroid reduction and discontinuation. Importantly, this trial was the first to establish the notion of the delayed onset of action, as the mean time to response was 3.1 mo, with 89% of responders doing so within 4 mo of starting mercaptopurine.

In the last two decades, all studies investigating the efficacy of thiopurines in inducing remission in CD have utilized active comparator groups rather than placebo alone. In a three-arm randomized, double-blind study comparing mercaptopurine 50 mg daily, oral methotrexate 12.5 mg weekly, and placebo in patients with active CD and Harvey-Bradshaw Index (HBI) ≥ 7, Oren *et al*[15] showed that the rates of remission (HBI ≤ 3 without steroids) using mercaptopurine or placebo were equivalent (9/32 in the mercaptopurine arm *vs* 6/26 in the placebo arm). This remission rate was not significantly different when compared to the methotrexate arm. In a similar study involving methotrexate, Maté-Jiménez *et al*[16], studied 38 patients with steroid-dependent CD who were randomized to mercaptopurine 1.5 mg/kg/d, methotrexate 15 mg/wk, or 5-aminosalicylic acid (5-ASA) 3 g/d. Compared with the 5-ASA group (14% remission), patients in both the mercaptopurine (93.7%) and methotrexate (80%) arms had statistically higher rates of remission. Finally,
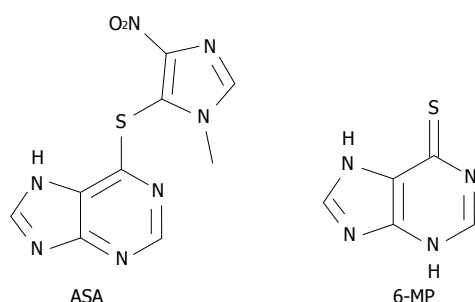
Figure 1 Chemical structures of azathioprine and mercaptopurine. AZA: Azathioprine; 6-MP: 6-mercaptopurine.

in a recent trial comparing azathioprine to methotrexate, patients with steroid-dependent CD with a CDAI ≥ 200 were treated with either intravenous methotrexate 25 mg/wk or oral azathioprine 2 mg/kg/d for a 6-mo period (in addition to a 12-wk prednisolone taper starting at 40 mg daily). The primary outcome - the proportion of patients entering first remission (CDAI < 150 without steroids) at 3 and 6 mo of therapy - was statistically similar between the two treatment groups (44% remission rate at 3 mo with methotrexate *vs* 33% with azathioprine; 56% remission rate at 6 mo with methotrexate *vs* 63% with azathioprine)[17].

A recent trial evaluating the effectiveness of thiopurines for the induction of remission in Crohn's disease was reported in 2010 by Colombel *et al*[18] in the Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease (the SONIC trial). The results of this study showed azathioprine to be less effective than infliximab as an induction agent for CD. Patients with active CD (CDAI 220-450) were randomized to one of three treatment arms: infliximab 5 mg/kg, azathioprine 2.5 mg/kg/d, or a combination of infliximab and azathioprine. Thirty-two percent (54/170) of azathioprine patients achieved clinical remission (CDAI < 150) at week 26 compared to 48% (81/169) of infliximab patients [risk ratio (RR) = 0.66, 95%CI: 0.51-0.87]. Similarly, significantly more infliximab patients than azathioprine patients achieved the primary study outcome of steroid free remission (44% *vs* 30%, respectively, *P* = 0.006). When assessing the combination of azathioprine and infliximab, significantly more patients in the combination therapy group (60%; 102/169) achieved clinical remission compared to patients treated with infliximab alone (48%) or azathioprine alone (32%, *P* < 0.001). Although, patients with heterozygous thiopurine methyl transferase (TPMT) activity were excluded, potentially minimizing the success of azathioprine monotherapy.

In addition, two randomized trials have found azathioprine therapy ineffective in achieving sustained corticosteroid-free remission. In an open-label trial of adults with CD for less than 6 mo at risk for disabling disease. patients randomly assigned to treatment with azathioprine 2.5 mg/kg/d were no more likely to experience clinical remission compared to patients who received azathioprine only in cases of corticosteroid dependency, chronic active disease with frequent flares, poor response to corticosteroids, or development of severe perianal disease[19]. In a prospective double-blind trial of patients with CD for less than 8 wk, patients randomly assigned to azathioprine 2.5 mg/kg/d were no more likely to achieve sustained corticosteroid-free remission compared to patients randomized to placebo (44.1% *vs* 36.5%), however, azathioprine was more effective in preventing moderate to severe relapse in a *post hoc* analysis[20].

The most recent Cochrane analysis (2013) evaluating the efficacy of thiopurines for induction of remission in CD compiled the results from 13 randomized control trials including 1211 patients: 9 comparing thiopurines to placebo and 6 using active comparators[21]. This analysis found no statistically significant difference in clinical remission rates between thiopurines and placebo (48% *vs* 37%, respectively, when combining the data from 5 studies with 380 total patients; RR = 1.23, 95%CI: 0.97-1.55). Thiopurine therapy was found to be no better at inducing steroid-free remission compared to methotrexate (RR = 1.13, 95%CI: 0.85-1.49) and 5-ASA or sulfasalazine (RR = 1.24, 95%CI: 0.80-1.91). Lastly, azathioprine was found to be significantly inferior to infliximab for induction of steroid-free clinical remission (RR = 0.68, 95%CI: 0.51-0.90). The only benefit of azathioprine for inducing remission in CD was found when it was used in combination with infliximab, as combination therapy was significantly superior to infliximab alone for induction of remission.

THIOPURINES FOR MAINTENANCE OF REMISSION IN CD

Dating back to the 1970s, multiple controlled trials have evaluated the efficacy of azathioprine and mercaptopurine for maintenance of remission in CD. While several earlier studies compared azathioprine or mercaptopurine to placebo, the more recent trials have used active comparators.

The first randomized, double-blind, placebo-controlled trial of azathioprine for therapy of CD was reported by Willoughby *et al*[14], in 1971. This small study aimed to determine the effect of azathioprine 2 mg/kg/d on maintaining remission of CD once it had been induced by prednisolone alone or in combination with azathioprine. At the end of the 24-wk study period, 4/5 patients on azathioprine maintained remission compared to 2/5 on placebo.

Over the course of the following three decades, multiple additional trials compared azathioprine to placebo[6,13,14,22-25]. Although these trials varied in their duration of therapy and dose of azathioprine (ranging from 1 mg/kg/d to 2.5 mg/kg/d), each added to the growing body of literature exploring the use of azathioprine for maintaining remission in CD. A

2015 Cochrane review presented a pooled analysis of the results from these seven studies[6]. In total, 532 patients were included, and there were a statistically higher proportion of patients who maintained remission over 6 to 18 mo with azathioprine compared to placebo. While only 58% (168/288) of patients on placebo maintained remission at study endpoints, 72% (175/244) of azathioprine patients were in remission (RR = 1.25, 95%CI: 1.11-1.42).

Two studies have compared azathioprine or mercaptopurine to 5-ASA or sulfasalazine for the maintenance of remission in CD. In 1979, Summers *et al*[13], reported the results from 86 patients with medically or surgically induced CD remission who were randomized to placebo, sulfasalazine, prednisone, or azathioprine (1 or 2.5 mg/kg/d). Over the course of one year, both patients in the azathioprine arm and sulfasalazine arm had similar rates of maintaining remission: 76% (53/73) azathioprine *vs* 68% (52/77) placebo. A second study by Maté-Jiménez *et al*[16] in 2000 reported the results from a maintenance trial in which CD patients in remission were randomized to either mercaptopurine 1 mg/kg/d or 5-ASA 3 g/d for 45 wk of therapy. Though mercaptopurine was superior to 5-ASA in maintaining remission (53% *vs* 0%), the numbers of patients in each study arm were very small. A pooled analysis of the results of these two trials found no difference between azathioprine or mercaptopurine and 5-ASA or sulfasalazine in the proportion of patients who maintained remission at 12 mo (RR = 1.09, 95%CI: 0.88-1.34)[6].

One study each has compared thiopurines to budesonide and methotrexate. In 2009, Mantzaris *et al*[24] suggested azathioprine (2.0-2.5 mg/kg/d) to be superior to budesonide (6-9 mg/d) in maintaining CD remission. In this prospective, randomized, controlled one-year trial including patients with steroid dependent CD in remission (CDAI < 150), 76% (29/38) of patients in the azathioprine arm maintained remission at one year compared to 46% (18/39) in the budesonide arm (RR = 1.65, 95%CI: 1.13-2.42). In contrast to these positive findings, Maté-Jiménez *et al*[16] found no difference in the rates of maintaining remission between mercaptopurine (50%; 8/16) and methotrexate (53%; 8/15) at 76 wk.

Finally, the most recent trial investigating the role of azathioprine in maintaining remission in CD utilized azathioprine in combination with infliximab and compared maintenance rates with infliximab alone[25]. In this study reported by Mantzaris *et al*[24], 47 patients with active, steroid-dependent CD were induced with tapered steroids along with infliximab 5 mg/kg at weeks 0, 2, and 6, or combination infliximab and azathioprine 2.5 mg/kg for 6 wk. Those entering remission were then continued into the maintenance phase of the trial where they were treated with either infliximab alone (5 mg/kg every 8 wk) or combination infliximab and azathioprine 2.5 mg/kg for 12 mo. The rates of maintaining remission at one year were statis-

tically similar in the combination therapy group (81%; 13/16) and the infliximab monotherapy group (80%; 16/20) (RR = 1.02, 95%CI: 0.74-1.40).

Given the cumulative results of the trials that have been conducted, a recent Cochrane review concluded that azathioprine is more effective than placebo for maintenance of remission in CD[6]. Similarly, azathioprine may be superior to budesonide for maintenance of remission, although this conclusion is based on the results of only one small study. Finally, thiopurines have not yet been rigorously compared to other active maintenance therapies, including infliximab, and more adequately powered trials are necessary to allow for definitive conclusions.

THIOPURINES FOR MAINTENANCE OF SURGICALLY INDUCED REMISSION IN CD

Approximately two thirds of CD patients will require at least one intestinal resection, and more than 50% will still require at least one additional surgery in their lifetime[23,26,27]. It is thus imperative to optimize post-operative prophylactic management strategies to reduce this risk. The role of thiopurines in post-operative prevention of CD recurrence will be discussed here.

A Cochrane analysis by Gordon *et al*[5] in 2014 embodies the most up to date, extensive review of thiopurine use as a preventative measure following intestinal resection. However, heterogenous study design by the trials in question makes the data less clear. Seven randomized controlled trials involving thiopurines were identified - four compared to 5-ASA[28-33], one compared to 5-ASA and adalimumab[34], one to infliximab[27], and one to placebo alone[35].

Thiopurine use overall appears to reduce post-operative recurrence risk when compared to placebo (12 mo endoscopic recurrence risk of 43.7% for azathioprine compared to 69% for placebo)[35]. However, efficacy outcomes when compared to 5-ASA agents were less clear, and did not demonstrate superiority of one modality over the other[28-30,33]. However, Reinisch *et al*[33] noted that perceived lack of efficacy may relate to suboptimal dosing strategies (with dosing based on metabolite levels providing better remission rates). Only two controlled trials addressed the utility of anti-TNF as compared to thiopurines, and results were conflicting[27,34].

Subsequent to the Cochrane analysis above, three randomized-controlled trials sought to examine the impact of anti-TNF therapy on post-operative disease recurrence, and indirectly addressed questions of thiopurine efficacy and optimization. Regueiro *et al*[36] randomized patients to either anti-TNF therapy or placebo after resection, and patients were instructed to otherwise continue pre-operative therapy. Forty-six

percent were taking a thiopurine throughout. Within the placebo arm, 100% of patients without thiopurine exposure experienced endoscopic disease recurrence compared to 71.4% of those on thiopurine mono-therapy, with a 28.6% reduction in recurrence ($P = 0.08$; the study was not powered to assess thiopurine efficacy). Of note, combination therapy of thiopurine and infliximab was not significantly more effective than infliximab monotherapy, with endoscopic recurrence rates of 27.8% and 18.5% respectively.

De Cruz *et al*[37] published data from the Post-Operative Crohn's Endoscopic Recurrence study in which patients at medium or high risk patients for postoperative recurrence of disease were then treated with adalimumab, thiopurine, or none of these agents. All patients received metronidazole initially and disease assessment followed with a "standard" or "active" assessment pathway, the comparison of which was the basis of the study[37]. Adalimumab did not appear significantly more effective at reducing short-term disease recurrence risk (43% endoscopic recurrence in adalimumab arm compared to 61% recurrence in mercaptopurine arm at 12 mo, $P = 0.17$). Notably, patients in the post-operative thiopurine who were on thiopurines prior to resection had similar outcomes to those who were pre-operatively thiopurine naïve, suggesting that "failing" a thiopurine preoperatively is not necessarily a contraindication for post-operative thiopurine use.

Finally, at the 2016 European Crohns and Colitis Organization annual meeting, initial data from the Trial of Prevention of Post-operative Crohn's Disease was presented; the largest double-blind placebo-controlled trial to date, comparing mercaptopurine to placebo for up to 36 mo postoperatively in 240 patients. The primary endpoint, CDAI evidence of recurrence, was reached in 23.2% of the placebo arm compared to 12.5% of the mercaptopurine arm (adjusted $P = 0.073$, unadjusted 0.046). Out to week 157, a higher proportion of mercaptopurine patients maintained endoscopic remission (Rutgeerts i0) than placebo (22.5% *vs* 12.5%, $P = 0.041$). In subset analysis, superiority of mercaptopurine over placebo was seen in smokers [hazard radio (HR) = 0.127, 95%CI: 0.04-0.46; number-needed-to-treat (NNT) = 3], but not in non-smokers.

Thiopurines appear to have a role in the prevention of recurrence of disease following intestinal resection. Consistently more effective than placebo, the utility of thiopurines when compared to mesalamine therapy is less clear, and may reflect a need to dose adjust according to serum thiopurine metabolite levels. The benefits of thiopurines use when compared to or in conjunction with anti-TNF would ideally be stratified in clinical practice according to the patient's risk of recurrence and prior management strategies.

THIOPURINES FOR INDUCTION OF REMISSION IN UC

While there are several randomized controlled studies regarding the efficacy of thiopurines in inducing remission in CD, there are considerably fewer high quality controlled studies in UC. The first controlled trials evaluating the effectiveness of thiopurines in UC were published in the 1970s[38,39]. In a small study of 20 patients with active proctocolitis, azathioprine 2.5 mg/kg/d produced significant improvement in clinical symptoms, inflammatory markers, and endoscopic and biopsy findings, but was not superior to sulfasalazine over a 3 mo period[38]. A similar study in 80 patients with flare of UC showed no benefit from the addition of azathioprine 2.5 mg/kg/d over 1 mo compared to a standard course of corticosteroids[39].

In a study by Sood *et al*[40], 83 patients with severe UC, steroid dependent on prednisone 1 mg/kg/d and sulfasalazine 6-8 g/d, were randomized to azathioprine or placebo with similar remission rates between aza-thioprine and placebo arms (68% and 64%, respectively). In a small trial on 34 patients with UC receiving prednisone for induction therapy, subjects randomized to additionally receive 1.5mg/kg/d of mercaptopurine were more likely to achieve steroid-free remission and a Mayo Clinic score less than 7 (78.6%) compared to patients randomized to additionally receive 3 g/d of 5-ASA (25%) over a 7.5 mo induction phase[16].

In an investigator-blind study by Ardizzone *et al*[41], 72 patients with steroid dependent, clinically and endoscopically active UC on prednisolone 40 mg/d were randomized to azathioprine 2 mg/kg/d or oral 5-ASA 3.2 g/d. At 6 mo, patients in the azathioprine group were more likely to experience corticosteroid-free, clinical and endoscopic remission compared to 5-ASA, both in intention-to-treat [odds ratio (OR), 4.78; 95%CI: 1.57-14.5] and per-protocol (OR = 5.26; 95%CI: 1.59-18.1) analysis[41].

In a meta-analysis of thiopurine induction therapy for remission of UC, the efficacy rate was 73% (95%CI: 63%-83%) for azathioprine and 64% (95%CI: 53-75%) for placebo or 5-ASA with an insignificant OR, of 1.59 (95%CI: 0.59-4.29)[42]. This efficacy rate decreased slightly to 65% (95%CI: 55-75%) based on large number of heterogeneous, open uncontrolled, and retrospective studies. Most of these studies, both controlled and uncontrolled, examined very small numbers of patients and differed significantly in study design, patient selection, classification of steroid dependence, thiopurine dosage, duration of induction, evaluation of response, follow up, and handling of concomitant treatments[42-46].

More recently, thiopurines are being positioned as part of combination therapy with TNF-α antagonists as induction therapy. In the UC SUCCESS randomized, double-blind trial of 239 patients with moderate to

severe UC, 39.7% (31 of 78) of patients receiving infliximab 5 mg/kg intravenous at weeks 0, 2, 6, and 14 with azathioprine 2.5 mg/kg/d, achieved corticosteroid-free remission at 16 wk compared with 22.1% (17 of 77) of patients receiving infliximab alone (*P* = 0.017) and 23.7% (18 of 76) of patients receiving azathioprine alone (*P* = 0.032)[47]. Rates of mucosal healing were also greater in patients exposed to combination therapy with infliximab and azathioprine compared to azathioprine monotherapy[47].

In lieu of the above studies, there is not clear evidence for the use of thiopurine monotherapy in UC induction, however, there may be evidence for thiopurines in combination with other immunosuppressive agents to improve the likelihood of inducing remission.

THIOPURINES FOR MAINTENANCE OF REMISSION IN UC

In one of the first controlled trials of azathioprine for maintenance, Jewell *et al*[39] (1974) followed 80 patients admitted for their first attack of UC, deriving no benefit from one-year maintenance with azathioprine 2.5 mg/kg/d[39]. Though azathioprine therapy appeared to reduce the relapse rate in patients who presented with flare of established UC, data failed to reach statistical significance.

In 1992, Hawthorne *et al*[48] examined the role of azathioprine in the maintenance of remission of UC in 67 patients and in 12 patients with active UC or corticosteroid dependence over the course of a year. Patients in remission and randomized to receive or continue azathioprine at a median dose of 100 mg/d had a relapse rate of 36% (12/33) compared with 59% (20/34) for patients given placebo (HR = 0.5; 95%CI: 0.25-1.0). After adjusting for sex, age, duration of remission and treatments prior to study entry, continued azathioprine therapy demonstrated a statistically significant benefit over withdrawal (HR = 0.43; 95%CI: 0.2-0.93)[48]. For patients with active disease at randomization, there was no benefit from continued azathioprine therapy[48].

In another study of 34 patients with UC, patients who achieved remission after induction with corticosteroids and maintained on mercaptopurine monotherapy for 76 wk were more likely to maintain remission (63.6%) compared to methotrexate (MTX) 15 mg/wk (14.3%) or 3 g/d of 5-ASA (0%)[16]. In a small study of 45 patients with steroid-dependent UC, patients randomized to sulfasalazine 6-8 g/d, oral prednisolone 1 mg/kg/d, and azathioprine 2 mg/kg/d had fewer relapses of disease over one year compared to sulfasalazine 6-8 g/d and oral prednisolone 1 mg/kg/d as corticosteroids were tapered over 12-16 wk[40].

In a similar study, 35 patients with newly diagnosed severe UC and randomized to sulfasalazine with azathioprine were significantly less likely to experience relapse of disease (4; 23.5%) compared to sulfasala-zine with placebo (10; 55.6%) over one year[49]. Moreover, maintenance of remission was significantly longer in patients given azathioprine with sulfasalazine compared to controls[49]. However, in a follow up prospective, randomized, open-label study from the same institution on 25 patients with severe UC on oral corticosteroids tapers, no difference was detected in relapse rates between patients given azathioprine 2.5 mg/kg/d compared to sulfasalazine 6 g/d[50].

There are several other observational, retrospective, and nonrandomized trials evaluating thiopurine therapy in UC maintenance therapy[41,51-57]. Data from a meta-analysis demonstrated an overall mean efficacy of 65% (95%CI: 62%-67%), with a mean efficacy of 66% (95%CI: 59%-73%) for steroid-resistant patients and 71% (95%CI: 66%-77%) for steroid-dependent patients[42]. In evaluating fairly homogenous controlled studies encompassing 124 patients, mean efficacy was 60% (95%CI: 51%-69%) for thiopurine therapy and 37% (95%CI: 28%-47%) for placebo and 5-ASA with an OR, of 2.56 (95%CI: 1.51-4.34) for maintaining remission[42]. The authors also calculated an absolute risk reduction of 23% with a NNT with thiopurines of 5 to prevent one relapse of disease[42].

According to a Cochrane analysis (2012) designed to assess failure to maintain clinical or endoscopic remission at 12 mo, based on 4 studies and 232 patients, there is low-quality evidence that azathioprine is superior to placebo for maintenance of remission in UC with a RR, of 0.68 (95%CI: 0.54-0.86)[2]. However, given varied treatment schedules, there was not enough evidence to determine an effect by dose or with combining medications. In this analysis, this pooled risk ratio suggested a NNT with azathioprine of 5 to prevent one relapse of disease, with an attributable risk reduction of 21%[2]. The authors of this analysis did not feel the existing data supported any meaningful evidence for thiopurines over 5-ASA agents or sulfasalazine, with an insignificant RR, of 1.52 (95%CI: 0.66-3.50)[2].

Overall, there is a lack of high quality trials evaluating the use of thiopurine therapy in maintenance of remission in UC. Existing evidence does not support thiopurine use alone or in combination with standard 5-ASA as compared to standard maintenance with 5-ASA therapies alone for remission in UC. Thiopurines may, however, be useful in patients who cannot tolerate 5-ASA therapies or in patients who require repeated courses of corticosteroids to induce remission. Considerably more data is required to further evaluate the use of thiopurines for maintenance of remission in UC compared to standard maintenance therapy, particularly in the era of biologics.

THIOPURINES IN CHEMOPREVENTION

In patients with IBD, chronic intestinal inflammation is a major risk factor for the development of gastrointestinal malignancy. In a meta analysis, quantitative

estimates of CRC risk in UC have been reported to be 2% after 10 years, 8% after 20 years, and 18% after 30 years of disease[58]. More recent data (2015) has found a cumulative risk of advanced neoplasia in UC of 2%, 5.3% and 14.7% at 10, 20 and 30 years, respectively[59]. In addition, numerous cohort studies on CRC in UC have noted a relationship between CRC risk with the extent of disease, with a standardized incidence ratio (SIR) of 1.7 for proctitis, 2.8 for left-sided colitis, and 14.8 for pancolitis[60]. In a population-based cohort study, patients with CD were also shown to be at an increased risk of CRC, with a pooled SIR of 1.9 (95%CI: 1.4-2.5)[61].

Although it has recently been suggested that the risk of CRC in IBD may be overestimated and more recent population-based studies in UC have demonstrated a decreasing risk of CRC over the past several decades, it is clear that colonic inflammation is a major risk factor for CRC[62]. Based on this risk, many studies have the examined the potential chemopreventive benefits of medications that reduce inflammation in IBD, with thiopurines being the most reported in the literature. However, there is conflicting data regarding the chemoprophylaxis effects of thiopurines on the risk of dysplasia and CRC in IBD.

The first study to address this question was published in 1994 from a prospective registry of 755 patients with IBD from the St. Mark's Hospital, London[63]. In this cohort, 15 patients with UC developed CRC with no significant modification by thiopurine exposure. In a larger study of 364 cases of CRC and 1172 matched controls, thiopurine use in the 12 mo preceding a diagnosis of CRC was not protective compared to unexposed controls (OR = 1.35, 95%CI: 0.92-1.98)[64]. In another retrospective study on 315 patients from the Mount Sinai Hospital, New York, who underwent surveillance colonoscopy, 96 (30.5%) were exposed to thiopurines for an average duration of 7.4 years at an average dose of 60.6 ± 19.5 mg/d of mercaptopurine equivalents. There was no protective effect of thiopurine exposure on colorectal neoplasia with 16% of patients exposed to thiopurines and 18% of those unexposed progressing to any neoplasia (HR = 1.06, 95%CI: 0.59-1.93) with 5% of patients in each group developing advanced neoplasia (HR = 1.30, 95%CI: 0.45-3.75)[65]. Similarly, in a study of 188 patients with UC-related colorectal cancer and matched controls, there was no association between thiopurine use and colorectal cancer (OR = 2.1, 95%CI: 0.7-7.2)[66]. Several other studies have produced similar findings, confirming a lack of benefit for thiopurines in preventing CRC[43,67-74].

More recent data, however, has suggested a potential role for thiopurines in chemoprevention. In a nationwide nested case-control study from a Dutch pathology database of 173 cases of IBD-related CRC and 393 matched IBD controls, patients treated with thiopurines were less often diagnosed with CRC compared with those never treated with thiopurines with an OR, of 0.36 (95%CI: 0.16-0.36)[75]. In a Dutch insurance-based cohort study of 2578 patients with IBD comprising 16289 person-years of follow-up, those who had used ≥ 50 mg of thiopurines per day for at least 6 mo had a significantly decreased risk of developing advanced neoplasia (adjusted HR = 0.10, 95%CI: 0.01-0.75)[76].

Further support for a protective effect of thiopurines was established using data from the ENEIDA registry (Estudio nacional en Enfermedad Inflamatoria Intestinal sobre determinantes genéticos y ambientales), a nationwide, hospital-based, prospectively maintained, Spanish database of incident and prevalent IBD patients[59]. In this study of 831 patients with UC with 26 cases of CRC and 29 cases of high-grade dysplasia, use of thiopurines (OR = 0.21, 95%CI: 0.06-0.74, *P* = 0.015) and being in a surveillance colonoscopy program (OR = 0.33; 95%CI: 0.16-0.67; *P* = 0.002) were independent protective factors for advanced neoplasia[59].

In a meta-analysis by Jess *et al*[4] based on two population-based studies and 13 Clinic- and insurance-based studies, there was no significant overall protective effect of thiopurines on colorectal neoplasia in IBD (OR = 0.87, 95%CI: 0.71-1.06). There was, however, a tendency toward a protective effect of thiopurines in studies using both colorectal dysplasia and CRC as the outcome instead of CRC alone (OR = 0.72, 95%CI: 0.50-1.05). In this analysis, a meta-regression suggested a trend toward a protective effect of thiopurines in more recent studies, but was not statistically significant (meta-regression; *P* = 0.16). Another meta analysis, however, based on nine case-control and ten cohort studies demonstrated that the use of thiopurines was associated with a significant decreased incidence of colorectal neoplasm in IBD (RR = 0.71, 95%CI: 0.54-0.94), even after adjustment for duration and extent of the disease[3].

Although the data is not overwhelmingly clear given the heterogeneity in the abovementioned trials, it is likely that there is some benefit to thiopurine therapy in reducing the risk of colitis-associated CRC in IBD. Future prospective studies would be useful to clarify if simply control of inflammation reduces the risk of CRC rather than a direct effect of thiopurines and the appropriate dosing and duration for maximizing a potential chemoprotective benefit.

THIOPURINE ADVERSE DRUG REACTIONS AND THE RISK OF INFECTION

It is well established that thiopurines are effective in treating IBD. This effectiveness, however, must be weighted against various adverse reactions with up to 60% of patients discontinuing thiopurine therapy during their disease course[43,77-81]. Multiple studies have cited intolerable dose-dependent and idiosyncratic

adverse events, such as hepatotoxicity, myelosupression and pancreatitis as primary reasons for discontinuation. Adverse effects tended to occur within the first three months of thiopurine initiation and longer duration of use appears to be associated with a lower risk of discontinuation[53,78,81]. In a Dutch cohort study of 363 patients over eight years of follow up, 32% experienced hepatotoxicity, 19% gastrointestinal effects, 12% myelosuppression, 11% pancreatitis, 11% fever, 9% general malaise, and 8% arthralgia[78].

Given that thiopurines have broad suppressive effects on the immune system, benign and opportunistic infectious complications are a serious concern to both patients and providers. This risk is further compounded in patients who require multiple immunosuppressive agents such as corticosteroids and biologic therapy. Studies have demonstrated increased rates of viral, fungal, parasitic, bacterial, and mycobacterial infections in patients exposed to thiopurine therapy[82,83].

Retrospective analyses of patients with IBD treated with thiopurines have showed rates of infection ranging from 7.4% to 14.1%[82,83]. Viral infections are of particular concern with a predisposition to cytomegalovirus, varicella zoster virus and Epstein-Barr virus (EBV) infections as a result of thiopurine induced T lymphocyte apoptosis[84-88]. In a study from the Mayo Clinic, thiopurine use increased the risk of an opportunistic infection by 2-3 fold (OR = 3.8, 95%CI: 2.0-7.0) and when combined with corticosteroids, greatly increased the risk (OR = 17.5, 95%CI: 4.5-68)[87]. Moreover, data from the Crohn's Therapy, Resource, Evaluation, and Assessment Tool registry found that the concomitant use of infliximab did not increase the risk of serious infection compared to conventional immunomodulator therapy alone over 2 years of follow-up[86].

Based on the above evidence, it is clear that the increased risk of serious infection associated with thiopurine use must be carefully balanced with the therapeutic benefits. This increased risk of infection requires appropriate prevention, prompt diagnosis, and effective treatment.

THIOPURINES AND THE RISK OF MALIGNANCY

In addition to the risk of infection, thiopurines also increase the risk of cancer. Thiopurines promote the development of cancer by a variety of mechanisms including direct alteration in DNA, activation of oncogenes, reduction in immunosurveillance of malignant cells, and impaired control of oncogenic viruses[89-91]. Several population-based cohort and meta-analyses have demonstrated that current use of thiopurines for IBD is associated with a 1.3 to 1.7 overall relative risk of cancer[71,92]. Specific cancers linked to long-standing thiopurine use in the setting of IBD include lymphomas, myeloid disorders, and skin cancers.

Multiple studies have demonstrated an increased risk of Non-Hodgkin Lymphoma following thiopurine exposure, with standardized incidence ratios ranging from 1.6 to 37.5, with no excess risk attributed to IBD itself[93-95]. The majority of lymphoma associated with thiopurine exposure is EBV-associated, resulting from the loss of immune control of EBV-infected B lymphocytes[96]. More concerning, there are several cases in the literature of fatal post-mononucleosis lymphoma in young men who previously tested seronegative for EBV[93]. Furthermore, Hepatosplenic T-cell Lymphoma, though extremely rare, is associated with thiopurine use in combination with TNF-α antagonists in adolescent and young males[97]. Despite the increased risks, recent data suggest no differences of survival with lymphoma between patients with IBD and expected survival for the general population[98].

In terms of myeloid disorders, in a study from the Cancers Et Surrisque Associé aux Maladies inflammatoires intestinales En France (CESAME) cohort, the risk of myeloproliferative disease was not increased among patients with IBD or ongoing thiopurine treatment (SIR = 1.54; 95%CI: 0.05-8.54)[92]. However, patients with past exposure to thiopurines had an increased risk of myeloid disorders (SIR = 6.98; 95%CI: 1.44-20.36)[92]. For skin cancers, there is considerable evidence that thiopurines increase the risk of basal cell and squamous cell carcinomas[99-101]. In another study from the CESAME cohort, an increased risk of basal cell and squamous cell carcinomas was demonstrated in the patients with IBD and associated with ongoing thiopurine exposure (HR = 5.9; 95%CI: 2.1-16.4) and past thiopurine exposure (HR = 3.9; 95%CI: 1.3-12.1)[101].

Although there is clear evidence for a risk of primary cancers associated with thiopurine use, several retrospective and prospective cohort studies have demonstrated no increased risk of new or recurrent cancer in patients with a history of cancer exposed to thiopurine therapy. Although data is limited, the CESAME group found that exposure to thiopurines did not increase the risk of new or recurrent cancer in patients with a history of cancer[92]. In a study by the New York Crohn's and Colitis Organization, exposure to TNF-α antagonists, antimetabolites (thiopurines or methotrexate), or the combination of these agents, was not associated with an increased risk of new or recurrent cancer within 5 years following a diagnosis of cancer (Log-rank P = 0.14)[102]. Furthermore, after adjusting for the risk of recurrence of prior cancer, there was still no difference in risk of new or recurrent cancer between exposure groups (anti-TNF-α HR = 0.32, 95%CI: 0.09-1.09; anti-TNF-α with an antimetabolite HR = 0.64, 95%CI: 0.26-1.59; antimetabolite HR = 1.08, 95%CI: 0.54-2.15)[102].

THIOPURINE DRUG METABOLISM AND BLOOD LEVEL MONITORING

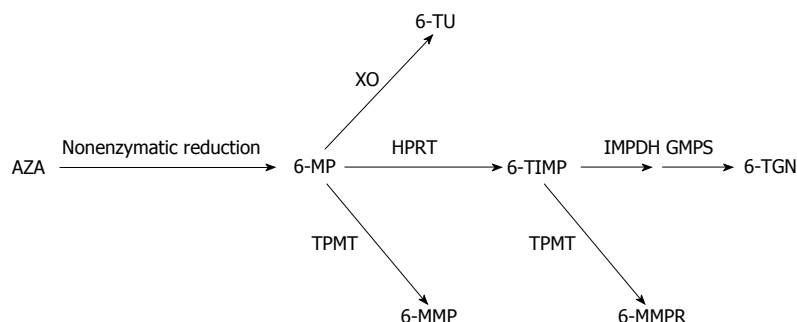Over the last decade, research has demonstrated that

Figure 2 Metabolic pathway for azathioprine and mercaptopurine. AZA: Azathioprine; 6-MP: 6-mercaptopurine; 6-TU: Thiouric acid; 6-MMP: 6-methylmercaptopurine; 6-TIMP: 6-thioinosine-monophosphate; 6-MMPR: Methyl-mercaptopurine ribonucleotide; 6-TGN: Thioguanine nucleotide; XO: Xanthine oxidase; TPMT: Thiopurine methyltransferase; HPRT: Hypoxanthine phosphoribosyl transferase; IMPDH: Inosine monophosphate dehydrogenase; GMPS: Guanosine monophosphate synthetase.

thiopurine efficacy is dependent upon a therapeutic blood value of 6-thioguanine nucleotide (6-TGN), the metabolic product of the parent drug[18]. Azathioprine is metabolized into 6-mercaptopurine in the liver by a non-enzymatic pathway[103]. After conversion into 6-mercaptopurine, different metabolic pathways compete, leading to the formation of 6-TGN by hypoxanthi-nephosphoribosyl transferase and 6-methylmercapto-purine by the TPMT enzymatic system (Figure 2). The therapeutic metabolite 6-TGN inserts itself into the DNA of leukocytes as a fraudulent base, thereby preventing T-cell proliferation, leading to subsequent immunosuppression[104]. In addition, studies have also demonstrated that azathioprine and its metabolites induce T cell apoptosis by modulation of Ras-related C3 botulinum toxin substrate 1 (Rac1) activation[105]. 6-methylmer-captopurine is associated with hepatotoxicity.

TPMT activity, ranging from the rare complete deficiency in 0.3% of adults to homozygous (normal) activity in 90% of adults, determines the breakdown to 6-methylmercaptopurine, the metabolic product causing hepatotoxicity. Prior to the assay for the presence of TPMT, the initial dose of mercaptopurine was 50 mg/d and then complete blood counts were followed with titration of the dose to the white blood count. With the advent of an assay for TPMT enzyme levels, it is now standard of care to measure its presence prior to initiation of therapy to identify patients at risk for toxicity. However, TPMT screening does not obviate the need for periodic hematologic monitoring.

Moreover, several studies have shown that 6-TGN levels greater than 235 pmol/8×10^8 red blood cells (RBCs) correlate with therapeutic efficacy[54,104,106,107]. This level is not weight based and although it is recommended that 6-TGN levels are monitored, especially in nonresponsive patients, many gastroenterologists initiate weight based dosing followed by dose titration dictated by clinical factors and leukocyte count.

The concomitant use of 5-ASA increases 6-TGN levels, improving therapeutic potential, however, the combination of these medications may also lead to greater risk of toxicity, especially myelotoxicity[108,109].

In patients with increased TPMT activity leading to high levels of 6-methylmercaptopurine and lower levels of 6-TGN, the addition of allopurinol has been shown to inhibit xanthine oxidase activity resulting in higher therapeutic 6-TGN levels and lower 6-methylmercap-topurine levels. In addition, allopurinol upregulates aldehyde oxidase and therefore 6-thioxanthine production, which then inhibits TPMT[110]. Several studies have reproduced these findings and dual therapy with allopurinol can improve the therapeutic effect and decrease hepatotoxicity[111,112]. Practically, the addition of 100 mg of allopurinol should lead to decreasing the thiopurine dose by 50% and complete blood counts must be followed closely for myelosuppression[111,113].

More recently, several studies have examined the impact of low-dose weight-based azathioprine in combination with allopurinol in patients with normal TPMT activity. In a small prospective cohort, 69.6% patients with IBD randomized to low-dose azathioprine in combination with allopurinol 100mg were in clinical remission without the need for steroid or biologic treatment, and with less adverse events, at 24 wk compared to 34.7% of the patients treated with aza-thioprine monotherapy[114]. In an uncontrolled, retro-spective, observational cohort of patients treated with low-dose weight-based azathioprine in combination with allopurinol, 69% with CD and 61% with UC had a clinical response at a median of 19 mo with 52% and 54% in clinical remission, respectively, with the highest response rates for thiopurine-naïve patients[115]. These studies suggest that low-dose weight-based azathio-prine in combination with allopurinol may be effective therapeutic strategy.

In patients treated with 5-ASA or allopurinol with thiopurines, periodic therapeutic drug monitoring is necessary in order to minimize toxicity. In addition, in patients intolerant to thiopurines secondary to preferential 6-methylmercaptopurine metabolism, it is possible to achieve therapeutic 6-TGN levels while reducing 6-methylmercaptopurine levels by splitting the dose or changing to 6-TGN as primary therapy[116].

COMBINATION THERAPY: THIOPURINES WITH TNF-α ANTAGONISTS

Studies have also demonstrated the importance of thiopurines in combination with anti-TNF therapies. In an ad hoc analysis of A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen (ACCENT I) trial, it was apparent that patients receiving concomitant immunomodulators had an improved remission and response rate at week 52[117]. This was the first indicator that combination therapy was more effective than monotherapy, likely attributed to immunomodulators decreasing antibody response to infliximab. Subsequently, numerous studies have supported initial evidence that immunogenicity contributes to increased formation of antibodies to anti-TNF, leading to lower trough levels, and eventual loss of response[118,119].

The effect of combination therapy in preventing antibody formation has been reproduced in various studies, including both the SONIC trial and UC SUCCESS[18,47]. Both of these trials compared infliximab or azathioprine monotherapy to combination therapy in patients naïve to both anti-TNF and thiopurine therapy. In both CD and UC, patients treated with combination therapy were more likely to achieve corticosteroid free remission, response, as well as mucosal healing, and there is now ample data to suggest that combination therapy is associated with higher anti-TNF trough levels[120]. In UC SUCCESS, 19% of patients receiving infliximab monotherapy developed antibodies *vs* 3% in patients receiving combination therapy[47]. However, sub-analysis of randomized control trials on anti-TNF use in patients who were not naïve to either an anti-TNF or thiopurine have failed to show an impact of combination therapy on clinical outcomes, despite less formation of antibodies. Similarly, Jones *et al*[121] studied the effect of adalimumab monotherapy *vs* combination therapy in patients not naïve to both drugs, and the data failed to show a higher remission or response rate with combination therapy at 52 wk. Similarly, in employing combination therapy with adalimumab, only a modest improvement was noted in those receiving combination therapy, with less need for dose escalation in this sub-group[122].

There have been various other trials examining the effect of combination therapy administered for 3, 6, or 9 mo with the suggestion that the initial combination decreases the long-term antibody formation thereby improving long-term clinical outcomes[123,124]. All of the aforementioned trials incorporated weight based azathioprine or mercaptopurine dosage.

A recent study assessed the correlation between levels of 6-TGN with both infliximab trough and antibody levels to infliximab[125]. Seventy-two patients who received combination maintenance therapy had levels of 6-TGN that significantly correlated with infliximab trough levels and antibody levels. Contrary to 6-TGN levels greater than $235 \text{ pmol}/8 \times 10^8$ RBCs, which is considered therapeutic under monotherapy, those treated with combination therapy only required a 6-TGN level of $125 \text{ pmol}/8 \times 10^8$ RBCs in order to decrease antibody formation and attain therapeutic levels of infliximab. This is the first study to suggest that prevention of antibodies to anti-TNF may require minimal doses of an immunomodulator, which theoretically may decrease long-term adverse effects.

Many observational trials have attempted to answer whether combination therapy is more effective than monotherapy. There are conflicting studies and henceforth, still some uncertainty as to whether and when to initiate thiopurines therapeutically as well as in preventing antibody formation. With the advent of more biologics and small molecules as therapy for IBD, thiopurines may be administered in smaller doses, and continue to play an integral role in maintaining therapeutic response.

EXPERIENCE OVERVIEW BY DRS. BURTON KORELITZ AND DANIEL PRESENT: INTERVIEWED BY DR. SIMON LICHTIGER

Induction of remission with thiopurines in CD can usually be achieved within 4 to 6 wk, with steroids serving as a bridge. For induction of remission in ulcerative colitis, although no randomized, placebo-controlled trial has confirmed their efficacy, we know well from our practice that thiopurines are effective and possibly work faster than in CD. For postoperative prevention, although our own studies did show statistical significance, the results are less robust than for anti-TNFs.

In lieu of enzyme testing or levels, we have always started patients on 50 mg per day of mercaptopurine and monitored the white blood cell count for leukopenia and the mean corpuscular volume for macrocytosis. Leukopenia would typically occur very quickly, so labs by week two would often detect these patients. Abnormalities in liver function are rare and we accept mildly elevated transaminases up to 200 with clinical efficacy. These laboratory values are also useful surrogates to assess medication compliance. For patients with mild side effects such as rash, fever, or arthralgia, we have found slow desensitization to be relatively simple and very effective starting with 1/8th the dose of mercaptopurine followed by a slow escalation. However, in patients with pancreatitis, desensitization is rarely successful and not recommended.

In our experience, opportunistic infections are rare and more often found in patients on concomitant steroids. We also feel that solid malignancies, such as breast, lung, liver, pancreas, and kidney are not more common in those treated with thiopurines. We had not even heard of hepatosplenic T-cell lymphoma until the advent of biologics. Dr. Present always felt his practice

Table 1 Current evidence and dosing for thiopurines in inflammatory bowel disease	
Thiopurine indication	Evidence and azathioprine dose
Crohn's disease induction	Monotherapy, less robust evidence: 1.5-2.5 mg/kg/d[11-17] Combination therapy with infliximab, more robust evidence: 2.5 mg/kg/d[18,19]
Crohn's disease maintenance	Monotherapy, more robust evidence: 1.5-2.5 mg/kg/d[6,13,14,22-25]
Postoperative maintenance in Crohn's disease	Monotherapy, more robust evidence: 2-2.5 mg/kg/d[5,28-36]
Ulcerative colitis induction	Monotherapy, less robust evidence: 1.5-2.5 mg/kg/d[16,38-42] Combination therapy with infliximab, more robust evidence: 2.5 mg/kg/d[47]
Ulcerative colitis maintenance	Monotherapy, less robust evidence: 2-2.5 mg/kg/d[2,16,40,42,50]
Chemoprevention	Monotherapy, less robust evidence: dose not established[3,4,59,74-76]
Preventing immunogenicity to anti-TNF	Combination therapy with anti-TNF, less robust evidence: dose not established[47,117-125]

TNF: Tumor necrosis factor.

did not demonstrate any increased risk of lymphoma with only 2 cases after more than 45 years of thiopurine use, but data demonstrates that lymphoma is statistically increased, albeit very rare.

CONCLUSION

Based on the above evidence and clinical experience overview by Drs. Korelitz and Present, thiopurines have demonstrated efficacy in a broad range of presentations of IBD (Table 1). Although extensive evidence for thiopurines has often been limited by trial methodology and design, over 50 years of clinical experience has demonstrated its efficacy and relative safety[126,127]. With the advent of more efficacious novel therapeutic agents with a wide variety of immunologic targets, the positioning of thiopurines in the management of IBD will undoubtedly change. Future studies will analyze the benefit of thiopurines in conjunction with these new medications, both as an individual synergistic adjunct and as a preventive agent for the production of antibodies to biologics. Notwithstanding, it continues to be a useful therapeutic option as monotherapy and in combination with other medications for inducing and maintaining durable remission in IBD.

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