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TOPIC HIGHLIGHT

2015 Advances in Inflammatory Bowel Disease

How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease?

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Abstract

In the last 15 years the management of inflammatory bowel disease has evolved greatly, largely through the increased use of immunomodulators and, especially, anti-tumor necrosis factor (anti-TNF) biologic agents. Within this time period, confidence in the use of anti-TNFs has increased, whilst, especially in recent years, the efficacy and safety of thiopurines has been questioned. Yet despite recent concerns regarding the risk: benefit profile of thiopurines, combination therapy with an immunomodulator and an anti-TNF has emerged as the recommended treatment strategy for the majority of patients with moderate-severe disease, especially those who are recently diagnosed. Concurrently, therapeutic drug monitoring has emerged as a means of optimizing the dosage of both immunomodulators and anti-TNFs. However the recommended therapeutic target levels for both drug classes were largely derived from studies of monotherapy with either agent, or studies underpowered to analyze outcomes in combination therapy patients. It has been assumed that these target levels are applicable to patients on combination therapy also, however there are few data to support this. Similarly, the timing and duration of treatment with immunomodulators when used in combination therapy remains unknown. Recent attention, including post hoc analyses of the pivotal registration trials, has focused on the optimization of anti-TNF agents, when used as either monotherapy or combination therapy. This review will instead focus on how best to optimize immunomodulators when used in combination therapy, including an evaluation of recent data addressing unanswered questions regarding the optimal timing, dosage and duration of immunomodulator therapy in combination therapy patients.

Key words: Inflammatory bowel disease; Thiopurines; Drug monitoring; Tumor necrosis factor-alpha; Combination therapy

Core tip: Clinicians managing inflammatory bowel disease frequently have to decide whether to use anti-tumor necrosis factor (anti-TNF) therapy alone or in combination with immunomodulators (IM), which requires an assessment of patient factors and the risk/benefit profile of each treatment strategy. Once a decision is made to use combination therapy, questions on how best to optimize IMs must be addressed. Thiopurines, rather than methotrexate, (MTX) are more efficacious and easier to administer, whereas in certain population groups, MTX may be safer. The effective dose of IM may be lower in combination therapy and combination therapy is probably most important in the first 12 mo of treatment. Withdrawing IMs is best done when the patient is in deep remission, ideally supported by the use of therapeutic drug monitoring of anti-TNFs.

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INTRODUCTION

Inflammatory bowel disease (IBD) namely Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions characterized by an exaggerated host immune response to an as yet unidentified antigen, leading to relapsing and remitting inflammation resulting in damage to the gastrointestinal tract. Despite access to an expanding therapeutic armamentarium with the arrival of gut-specific therapies such as vedolizumab and other novel agents targeting key pro-inflammatory cytokines, clinicians still largely rely on the conventional immunomodulators, (IMs) azathioprine, (AZA) mercaptopurine, (MP) and methotrexate, (MTX) and/or anti-tumor necrosis factor (anti-TNF) therapy, (infliximab, (IFX) adalimumab, (ADA) certolizumab pegol and to a lesser extent, golimumab) to treat these diseases. Much has been learnt over the last 15 years of the relative risks and benefits of using these agents, either alone or in combination, however gaps in our knowledge remain as to how IMs are best optimized once a decision has been made to combine them with anti-TNF therapy. This review article begins with a brief outline of the efficacy and safety issues surrounding combination therapy (IM + anti-TNF) and then draws on the available evidence to address some of these unanswered questions (Table 1).

Table 1 Summary and key points

Combination therapy (thiopurines with anti-TNF) is more efficacious than either agent alone in thiopurine-naïve patients with IBD
Combination therapy confers an increased risk of adverse events, of which NMSC, melanoma and lymphoma are the best studied
The benefit of combination therapy is probably due to both an improvement in anti-TNF pharmacokinetics (reduced immunogenicity and improvement in drug levels) and an independent effect of the IM on disease activity
The pharmacokinetic benefits of combination therapy are most important during the first 12 mo of therapy, but may persist beyond this
The optimal dose of IM in this setting may be lower than that used for IM monotherapy, however further studies are needed to confirm this
The risk of relapse after IM withdrawal is highest amongst patients with active disease and positive biomarkers of inflammation or unfavorable anti-TNF pharmacokinetic profiles
Withdrawal of IM should be considered in patients in deep remission after a period of 12 (or perhaps 24 mo) of combination therapy

TNF: Tumor necrosis factor; IBD: Inflammatory bowel disease; IMs: Immunomodulators; NMSC: Non-melanoma skin cancer.

BENEFITS OF COMBINATION THERAPY *VS* ANTI-TNF MONOTHERAPY

The arrival of IFX, and subsequently ADA, both effective therapies for induction and maintenance of remission for luminal and fistulizing CD and UC, revolutionized the management of IBD[1-9]. A common issue faced by clinicians is under what circumstances does combination therapy with an IM offer benefit over anti-TNF monotherapy. Amongst IM naïve patients with moderate-severe CD, the SONIC study (508 treatment naïve CD patients randomized to AZA, IFX or combination therapy) showed that combination therapy was superior to IFX monotherapy with respect to corticosteroid-free clinical remission (56.8% *vs* 44.4%, $P = 0.02$) and mucosal healing (43.9% *vs* 30.1%, $P = 0.06$)[10]. Similar results in moderate-severe UC were seen in the UC-SUCCESS trial, favoring combination therapy (AZA + IFX) over IFX monotherapy for clinical remission, (39.7% *vs* 22.1%, $P = 0.017$) and complete mucosal healing, (29.5% *vs* 11.7%, $P = 0.006$) at week 16[11]. These results should be interpreted with caution as this study was terminated early, and therefore underpowered, and week 16 may be too early for thiopurines to be efficacious; however combination therapy was as effective as, or superior to, IFX monotherapy across a range of secondary endpoints. COMMIT, a 50 wk randomized placebo-controlled trial of CD patients initiated on prednisolone found no benefit of MTX and IFX combination therapy ($n = 63$) over IFX monotherapy ($n = 63$) for the primary endpoint, defined as failure to enter steroid-free clinical remission at week 14, (78% *vs* 76%, P = NS) or failure to maintain remission through week 50, (57% *vs* 56%, P = NS)[12]. When reconciling the opposing findings of combination therapy *vs* anti-TNF monotherapy of SONIC/SUCCESS *vs* COMMIT, several

key differences in study design should be considered. COMMIT used a high dose corticosteroid induction regimen that may have obscured a true benefit of MTX combination therapy over IFX monotherapy. Further, the primary end-point of corticosteroid free remission may have been seen equally between treatment arms due to the enrolment of patients with milder CD activity, a proportion of which may have never failed treatment according to clinical (CDAI) criteria. Of note, in COMMIT, patients randomized to combination therapy had higher median trough drug levels compared to IFX monotherapy (6.35 μg/mL *vs* 3.75 μg/mL, P = 0.08), suggesting a beneficial effect of combination therapy on IFX pharmacokinetics.

Sub-group analyses of RCTs of IFX and ADA for both CD and UC, stratified according to baseline IM use, have failed to show a benefit of combination therapy over anti-TNF monotherapy in achieving clinical remission[1,4,6,7,9,13]. However, a large percentage of patients entered these studies already failing IMs, a key difference from the low proportion of previous IM use in SONIC, SUCCESS and COMMIT. Further, in the ADA RCTs there were high rates of previous IFX failure, (CHARM 49%[6], ULTRA-2 41%[9]) therefore these patients may represent a more treatment-refractory cohort. Data from observational studies has been conflicting with some supporting combination therapy over anti-TNF monotherapy[14-19], whereas others do not[20-23]. Differences in study design; patient populations and endpoints all hamper the strength of conclusions that can be drawn from these studies.

A post-hoc analysis of patient level data, (published in abstract form only) taken from 11 anti-TNF RCTs (IFX, ADA, and certolizumab pegol) found that combination therapy was more efficacious than monotherapy for 6 mo clinical remission in those treated with IFX (OR = 1.79; 95%CI: 1.06-3.01) but not ADA (OR = 0.88; 95%CI: 0.58-1.35) or certolizumab (OR = 0.93; 95%CI: 0.65-1.34)[24]. This may be explained as IFX, a chimeric anti-TNF is more immunogenic than the humanized ADA. A "SONIC-type" study comparing ADA monotherapy to ADA+IM combination therapy is needed before we can say with certainty that combination therapy is more efficacious in this setting.

Taken together the literature suggests that in IM naïve patients with moderate to severe IBD, combination therapy is more efficacious and should be considered over monotherapy with an anti-TNF, and that in IM refractory patients, combination therapy may be important for at least the first 12 mo of anti-TNF treatment.

RISKS OF COMBINATION THERAPY *VS* MONOTHERAPY

Infections and malignancy

Any putative increase in efficacy through the use of combination therapy must be balanced against the risk of adverse events, and infectious complications and malignancy in particular[25]. Randomized controlled trials in IBD have shown no significant increase in infections in patients treated with combination therapy compared with anti-TNF monotherapy. A pooled analysis of 1383 patients, randomized to receive either placebo or IFX, of which 40% received concomitant immunomodulation with AZA, MP or MTX from the landmark ACCENT Ⅰ and ACCENT Ⅱ (luminal and fistulizing CD respectively), and ACT Ⅰ and ACT Ⅱ (UC), studies showed similar rates of both infections (44.1% *vs* 44.5%) and serious infections (3.7% *vs* 3.2%) in those treated with immunomodulator co-therapy *vs* those treated with IFX monotherapy[26]. Similarly, in SONIC serious infections were seen in 4.9% *vs* 3.9%, (P = 0.79) of those treated with IFX monotherapy and combination therapy, respectively[10]. In COMMIT, respiratory infections occurred in 46% of patients treated with combination therapy compared with 41.3% of those treated with IFX (P = NS), although all patients also received an induction course of corticosteroids which may have contributed to these very high infection rates[12]. Despite these reassuring findings it must be emphasized that follow-up of these trials was relatively short (generally limited to 52 wk), and they were underpowered to detect uncommon opportunistic infections. Retrospective observational studies have reported conflicting infectious complication rates in anti-TNF monotherapy compared with combination therapy. Osterman and colleagues found an increased rate of opportunistic bacterial and fungal infections (HR = 2.64; 95%CI: 1.21-5.73) and herpes zoster (HR = 3.16; 95%CI: 1.25-7.97) amongst 577 patients who "stepped up" to ADA or IFX from IMs (92% thiopurines) over a median follow-up of 1.4-1.7 years, but no increase in the rate of serious infections amongst combination therapy compared with anti-TNF monotherapy[27]. Other studies have shown no increase in infections amongst combination therapy compared with anti-TNF monotherapy[28]. Despite these conflicting data on infection rates, an unequivocal signal from randomized controlled trials and observational studies is that corticosteroids impart a significant additive infective risk for both anti-TNF monotherapy and combination therapy exposed patients[29,30].

MALIGNANCY

It is accepted that thiopurines are associated with an increased risk of non-melanoma skin cancer, (NMSC) (basal cell carcinoma and squamous cell carcinoma) in post-transplant recipient patients[31]. Three large observational studies have demonstrated that thiopurine therapy confers a 4-6 fold increase in NMSC amongst patients with IBD and that this risk remains elevated compared to age-matched thiopurine naïve

patients with IBD even after stopping thiopurines[32-34]. In IBD there are no well-designed studies assessing the risk of NMSC in anti-TNF monotherapy, primarily because of confounding due to prior or concomitant thiopurine exposure. A meta-analysis of anti-TNF monotherapy use amongst patients with rheumatoid arthritis demonstrated an increased risk of NMSC (1.45, 95%CI: 1.15-1.76)[35]. In a nested case-control claim database amongst 3288 matched IBD patients, (3288 NMSC matched to 12945 controls) sub-group analysis of patients with ⩾ 1 year drug use demonstrated the greatest risk amongst combination thiopurines and anti-TNF, (adjusted OR = 3.89, 95%CI: 2.33-6.46) compared to thiopurine monotherapy (adjusted OR = 2.72, 95%CI: 2.27-3.26) and anti-TNF monotherapy (adjusted OR = 1.63, 1.12-2.36)[34]. Amongst patients with less than 12 mo anti-TNF use there was no association with NMSC. A pooled analysis of 1594 CD patients who participated in the landmark RCTs of ADA demonstrated no increased risk of NMSC in ADA monotherapy, compared with an increased risk of NMSC, and other malignancies, in thiopurine combination therapy (adjusted RR = 4, 95%CI: 1.23-13.0)[36]. Taken together, these results suggest that combination therapy increases the risk of NMSC above and beyond the risk of both thiopurine and anti-TNF monotherapy. Despite an apparent increased risk of melanoma amongst patients with IBD[34,37], thiopurine use does not seem to increase the risk further[34]. Anti-TNF therapy, in contrast, appears to double the risk of melanoma[34]. Similar associations between anti-TNF use and melanoma in RA have been observed[35,38,39]. As with NMSC, drawing firm associations between anti-TNF monotherapy exposure and melanoma risk are limited by current or past exposure to IMs.

Determining the influence of IM monotherapy *vs* combination therapy on lymphoma development is difficult due to the relatively uncommon occurrence of this event and the short follow-up period of RCTs. Pooled data from 7054 IBD patients from 11 RCTs, (IFX, ADA, certolizumab and golimumab) followed for 1 year, showed no cases of lymphoma amongst anti-TNF treated patients, compared to 3 placebo arm patients, (although 2 of these had received induction with anti-TNF)[40]. Other pooled analyses have demonstrated an increased risk of lymphoma with combination therapy, however these have not detected cases of lymphoma amongst those treated with anti-TNF monotherapy. This limits the strength of conclusions on the risk of lymphoma development between the two treatment strategies. Accordingly, data from large population-based observational cohort studies must be considered. In CESAME, a prospective observational cohort study of 19 486 IBD patients, the risk of lymphoma was higher amongst patients using thiopurines in combination with anti-TNF compared to thiopurines alone, [standardized incidence ratio, (SIR) = 10.2, 95%CI: 1.24-36.9, $P < 0.04$] *vs* 6.53, 95%CI: 3.48-11.2, $P < 0.0001$, respectively)[41]. Anti-TNF monotherapy did not increase the risk of lymphoma, (SIR = 4.5, 95%CI: 0.6-16.4, $P = 0.1$). Similarly a retrospective cohort study of 36891 Veteran Affairs UC patients, of which 4734 were treated with thiopurines for one year found an increased risk of lymphoma amongst thiopurine users (HR = 4.2, 95%CI: 2.5-6.8, $P < 0.001$)[42]. Subgroup analysis demonstrated a non-significant increased incidence rate ratio, (IRR) amongst thiopurine/IFX combination therapy (IRR = 3.84, 95%CI: 0.8-44.2) compared with thiopurine monotherapy (IRR = 3.6, 95%CI: 2.2-6.0) however only 1 case of lymphoma was diagnosed in the combination group, implying this study was underpowered to detect a true difference. The findings from other studies have been conflicting[27,43-48]. In general, observational studies and meta-analyses have shown that combination therapy increases the risk of lymphoma, however the magnitude of this risk is similar to that seen with IM monotherapy.

UNANSWERED QUESTIONS REGARDING THE OPTIMIZATION OF IMMUNOMODULATORS WHEN USED AS COMBINATION THERAPY

Which immunomodulator should be used - thiopurines or methotrexate?

The evidence as to which IM, thiopurines or MTX, to choose in combination therapy is limited, although there are more data relating to the use of thiopurines. Randomized controlled trials (RCTs) in both CD (SONIC)[10] and UC (SUCCESS)[11] demonstrate superiority of thiopurine-based combination therapy over anti-TNF monotherapy. In contrast, combination therapy with MTX has not been proven to be superior to monotherapy in CD (COMMIT)[12], and there are a lack of high quality data to support the use of MTX in UC when given as monotherapy, with no combination therapy data available[49]. However, given differing trial designs and endpoints, direct comparison of these RCTs must be interpreted with caution.

The benefits of adding an immunomodulator to anti-TNF therapy, even in patients who have previously failed immunomodulators, are presumably due to both a reduction in immunogenicity with a resultant increase in serum anti-TNF levels, and also a direct effect in reducing disease activity. Both thiopurines and MTX have beneficial effects on the pharmacokinetics of anti-TNF agents when used in combination therapy. In a retrospective, single-centre study of 174 CD patients treated with episodic IFX, AZA and MTX were equally effective in preventing immunogenicity (antibodies to IFX, (ATIs) 48% in AZA group *vs* 44% in MTX group, P = NS) and infusion reactions (18% *vs* 14% in AZA and MTX groups respectively, P = NS), and in increasing serum IFX levels (6.15 μg/mL *vs* 5.65 μg/

mL in AZA and MTX groups respectively, *P* = NS)[50]. The presence of ATI was associated with a shorter duration of response in patients not taking IM (median 11.7 wk) as compared to those taking IM (median 13.8 wk, *P* = 0.006) although numbers were small. In SONIC, patients on combination therapy with AZA had significantly higher IFX levels than monotherapy patients at week 30 (3.5 μg/mL *vs* 1.6 μg/mL, *P* < 0.0001)[10]. In the COMMIT study patients on combination therapy with MTX had lower rates of ATI formation than monotherapy patients (4% *vs* 20%, *P* = 0.01) and a trend to higher serum IFX levels (6.35 μg/mL *vs* 3.75 μg/mL, *P* = 0.08)[12].

Another advantage of thiopurines is the oral route of administration, compared to MTX, where only parenteral monotherapy in CD has been consistently demonstrated to be effective[51,52]. If used in therapeutic doses in combination therapy, presumably parenteral MTX is the best option. However if used primarily to reduce immunogenicity then rheumatologic data suggests that low dose oral MTX may be adequate. Published only in abstract form, it was demonstrated that the addition of MTX to maintenance ADA increased ADA levels from 5 μg/mL to between 8-9 μg/mL[53]. More recently in the CONCERTO trial 395 RA patients were randomized to open-label ADA 40 mg alternate weekly, and blinded oral MTX at doses or 2.5, 5, 10 and 20 mg weekly. ADA serum concentrations increased with increasing MTX doses up to 10 mg weekly, above which there was no dose response. Anti-adalimumab antibody prevalence was also similar between the 10 and 20 mg MTX groups, suggesting that in RA patients 10 mg MTX orally weekly is the correct dose to optimize ADA pharmacokinetics[54]. Whether these data are applicable to IBD is unknown. Similarly, thiopurines have consistently been shown to increase serum anti-TNF levels when given as combination therapy[10,55], although there are no data delineating an optimal weight-based thiopurine dose needed to achieve maximal serum anti-TNF concentrations.

Another consideration in the choice of concomitant immunomodulator is the small, but real, increased risk of lymphoma associated with thiopurines in IBD. The most recent meta-analysis of both population and referral-based IBD studies demonstrated a SIR of lymphoma of 4.92 (95%CI: 3.10-7.78) amongst thiopurine-exposed patients. The risk was highest amongst males currently receiving thiopurines for at least one year[48]. A similar increased magnitude of risk has been demonstrated in other recent population-based studies and meta-analyses[41,44]. Of particular concern is the association between thiopurine use and hepatosplenic T cell lymphoma (HSTCL), especially in young males under 35 years of age[56]. By contrast there are no studies showing an increased risk of lymphoma with MTX use in IBD, although it must be recognized that this is largely due to a lack of data rather than there being studies definitively showing no association. Studies in rheumatoid arthritis show conflicting data as to whether MTX use is associated with an increased lymphoma risk, either as monotherapy or when combined with anti-TNF agents[57-59]. In considering these data it would seem reasonable to consider MTX as the immunomodulator of choice when lymphoma risk is highest, such as in young males, whereas for other patients the benefits of thiopurines will usually outweigh the small lymphoma risk. Finally MTX is teratogenic and is contraindicated during pregnancy. Due to its long half-life it is recommended to stop MTX 3-6 mo pre-conception in females[60]. Its effects on male fertility and spermatogenesis are controversial; some experts recommend withdrawal in males 3 mo prior to trying to conceive[60].

When should immunomodulators be commenced when used as combination therapy?

The SONIC study demonstrated in a randomized controlled trial that clinical and endoscopic remission occurs most frequently when immunomodulators and IFX are commenced simultaneously in treatment-naïve patients[10]. Pharmacokinetic data from observational single-centre studies has subsequently emerged to support this practice.

In a retrospective study of 217 patients on anti-TNF therapy (108 IFX, 109 ADA) concomitant IMs improved pharmacokinetic outcomes for patients on IFX (83.1% thiopurines, 16.9% MTX), but not ADA (83.3% thiopurines, 16.7% MTX). For IFX, trough levels were significantly higher in the combination therapy group compared to monotherapy patients (7.5 μg/mL *vs* 4.6 μg/mL, *P* = 0.04), while for ADA no difference was seen (13.1 μg/mL *vs* 11.5 μg/mL respectively, *P* = 0.5). Similarly, combination therapy patients were less likely to have ATIs than monotherapy patients for IFX (5.7% *vs* 29.8%, *P* = 0.001), but not ADA (17.2% *vs* 21.6%, *P* = 0.6). Regarding the timing of introduction of the IM, IFX patients in whom IMs were started at the same time as the anti-TNF were less likely to develop ATIs than patients in whom IMs were started later (2.4% *vs* 18.2%, *P* = 0.04); again no difference was seen in ADA patients. Interestingly, there was no association between IM dose and IFX trough levels, and in fact counter-intuitively patients with suboptimal IM doses had higher trough levels (9.81 *vs* 5.36, *P* = 0.02). This study suggests that immunogenicity occurs early in the treatment course of anti-TNFs and that perhaps a lower dose of IM may be sufficient to prevent anti-drug antibody formation and optimize trough levels[61]. It is important to note that this pharmacokinetic study did not assess clinical outcomes, hence it is unclear whether the favorable effect of combination therapy on improving drug levels and reducing ATIs conferred a clinical benefit. Consistent with these results, in a prospective observational study of 125 patients treated

with IFX (98 CD, 27 UC), 46% of patients developed ATIs. Of these, 90% of patients who developed permanent ATIs did so within 12 mo of starting IFX, whilst transient, and clinically non-significant, antibodies developed at any time during therapy (P < 0.001). Patients on combination therapy had a longer ATI-free survival compared to monotherapy patients (P = 0.003, log rank test)[17]. Low IFX trough levels and high ATI titers were significantly more prevalent amongst patients with clinical loss of response, P < 0.001. These data therefore also demonstrate that IMs are most effective at reducing immunogenicity in the first 12 mo of anti-TNF therapy, suggesting that the two classes of therapy should be commenced simultaneously.

What dose of immunomodulator should be used when used as combination therapy - are lower doses equally effective and safer?

To date most studies of combination therapy have used full weight-based thiopurine doses (AZA-2.0-2.5 mg/kg per day, MP-1.0-1.5 mg/kg per day), with or without further dose-optimization aiming for therapeutic metabolite levels [6-thiogunanine nucleotide, (6-TGN) 235-450 pmol/8 × 10^8 RBC]. However, more recently, definite signals of thiopurine toxicity have been confirmed in large population-based studies, in particular the risk of infections, NMSC and lymphoma[32,41]. Of these adverse events, infection risk is definitely dose-dependent, however most population-based studies of NMSC and lymphoma risk have not included thiopurine doses in their analyses[32,48]. This raises the question of whether lower thiopurine doses can be used in combination therapy with equal efficacy and pharmacokinetic benefits on serum anti-TNF levels, and presumably, less toxicity. Recent retrospective and observational studies have explored the effect of thiopurine dose on outcomes when used in combination therapy, analyzing by mg/kg daily doses or surrogate measures of 6-TGN levels and changes in mean corpuscular volume (MCV) in thiopurine-treated patients.

In the Dutch retrospective study assessing pharmacokinetic outcomes of combination therapy (predominantly with thiopurines) there was no correlation between IM dose and anti-TNF levels, suggesting that lower IM doses in combination therapy may be equally effective[61]. More recently, in a single centre cross-sectional study of 72 patients (45 CD, 27 UC) on combination therapy with scheduled maintenance IFX and thiopurines, thiopurine metabolite levels were correlated with IFX levels and ATIs. There was a moderate correlation between 6-TGN concentrations and IFX levels (rho - 0.53, P < 0.0001). The 6-TGN cut off that best predicted higher IFX levels was 125 pmol/8 × 10^8 RBCs (AUROC - 0.86, P < 0.001). Patients with 6-TGN levels below this cut off had IFX levels similar to patients on monotherapy (4.3 μg/mL *vs* 4.8 μg/mL, P = 0.8). Similarly, patients with 6-TGN levels below this threshold were more likely to have ATIs (OR = 1.3, 95%CI: 2.3-72.5, P < 0.01). These results provide the first signal that lower thiopurine doses, as measured by metabolite levels, may be equally effective as therapeutic doses in optimizing serum anti-TNF levels, however they must be interpreted with caution. The primary endpoint was IFX levels, with mucosal healing as a secondary endpoint, and IFX levels of > 8.3 μg/mL were associated with mucosal healing. When dichotomized above and below this cutoff, a mean 6-TGN level of 223 pmol/8 × 10^8 RBCs was required to achieve an IFX level of 8.3 μg/mL, compared to mean 6-TGN levels of 128 pmol/8 × 10^8 RBCs for IFX levels < 8.3 μg/mL (P < 0.001). Similarly, undetectable *vs* detectable ATIs were associated with mean 6-TGN levels of 117 pmol/8 × 10^8 RBCs and 193 pmol/8 × 10^8 RBCs respectively (P = 0.024). Therefore, while a 6-TGN level of 125 pmol/8 × 10^8 RBCs best predicted increased IFX levels, very similar 6-TGN levels were associated with a lack of mucosal healing and the development of ATIs - this disparity may in part be explained by the high IFX cut off of 8.3 μg/mL that was used, for which sensitivity and specificity were only moderate (71% and 73% respectively)[62]. Similar findings were observed in a single centre cross-sectional study of 269 IBD patients treated with IFX who underwent TDM with a drug-tolerant mobility shift assay[63]. Patients co-treated with AZA/MP, [n = 99 (37%)] and MTX [n = 32 (12%)] were more likely to have therapeutic IFX levels than those on monotherapy, (P = 0.05 and P = 0.04 for thiopurines and MTX, respectively). Regression analysis did not demonstrate a relationship between AZA dose and drug levels (P = 0.88) nor was an association seen between weight based dose (mg/kg) and drug levels when analysed by quartiles (P = 0.87).

The change in MCV with thiopurine therapy has been correlated with 6-TGN levels, with a delta MCV of at least 7 fL being associated with therapeutic 6-TGN levels and improved clinical outcomes[64,65]. A post hoc analysis of the SONIC study [which included only patients with normal thiopurine methyltransferase, (TPMT) activity] investigated the relationship between the change in MCV (dichotomized to above and below 7 fL) and outcomes in patients receiving combination therapy with AZA and IFX. An increase in MCV of at least 7 fL was associated with mucosal healing at week 26 (75% *vs* 47.1% if delta MCV < 7 fL, P = 0.02) and IFX levels > 3.0 μg/mL (68.4% *vs* 38.8% if delta MCV < 7 fL, P = 0.003). On multivariate analysis, delta MCV > 7 fL was associated with mucosal healing (OR = 3.86, 96%CI: 1.05-14.19, P = 0.04). Interestingly, patients with a delta MCV > 7 fL had less infectious adverse events (26.5% *vs* 49.2% if delta MCV < 7 fL, P = 0.008). No correlation between changes in MCV and mg/kg thiopurine doses was performed and thiopurine

metabolites were not measured[66]. These results represent progress in optimizing thiopurines when used in combination therapy, although the optimal mg/kg dose, or surrogate measure of efficacy, remain to be determined.

Similarly, for MTX there are few data to guide clinicians as to the optimal dose, and route, to use in combination therapy with anti-TNF agents in IBD. In rheumatoid arthritis, 10 mg MTX orally weekly was the optimal dose to increase serum adalimumab levels in a MTX dose-escalation study[54]. In the COMMIT study subcutaneous MTX was commenced at 10 mg weekly and increased to 25 mg weekly by week 5, with the mean MTX dose at week 50 being 22.3 mg. At this dose, combination therapy patients compared to monotherapy patients had less ATIs (4% *vs* 20%, $P = 0.01$), numerically higher IFX trough levels (6.35 μg/mL *vs* 3.75 μg/mL, $P = 0.08$) and were more likely to have detectable IFX trough levels (52% *vs* 44%, $P = 0.84$). Even at this high dose, there was no difference in adverse event rates between the two groups[12]. More recently, in a single referral-centre retrospective study of combination MTX and anti-TNF therapy, outcomes were compared between patients on low dose (< 12.5 mg weekly) and high-dose (15-25 mg weekly) MTX. 73 IBD patients with active disease were included (CD-54, UC-16, indeterminate colitis - 3), of which 71% received high-dose and 29% low-dose MTX. The anti-TNF was ADA in 49% of patients, IFX in 40% of patients and certolizumab in 11% of patients, and MTX was given orally in 75% of patients. 46 of 73 (62%) patients went into remission and were followed and included in the primary analysis of duration of remission maintenance. High-dose MTX combination therapy patients were less likely to relapse (log-rank test, $P < 0.01$), and although rates of adverse events (33% *vs* 12%, $P = 0.13$) and discontinuations (14% *vs* 6%, $P = 0.34$) were higher in the high-dose MTX group, these differences did not reach significance. There were no differences when analyzed by the anti-TNF used in combination therapy (log-rank test, $P = 0.58$), diagnosis (log-rank test, $P = 0.78$), or mode of MTX administration (log-rank test, $P = 0.56$). Therapeutic drug monitoring was not performed[67].

Although a lower dose of concurrent IM would be hoped to be safer, in particular resulting in fewer infections and malignancies, there are few data to support this assumption. Studies amongst non-IBD populations have found a relationship between rates of malignancy and total thiopurine dose, thiopurine metabolite levels and TPMT activity[68-70]. Caution must be exercised before extrapolating these findings to the setting of combination therapy in IBD. Thiopurines are associated with increased infections, and viral infections in particular, (as outlined above) although a post-hoc analysis did not find a difference in infection risk between patients on high dose *vs* low dose thiopurines[27]. Similarly, the risk of NMSC and lymphoma associated with thiopurines has never been demonstrated to be dose-dependent in IBD, however most studies addressing these questions have not included IM dose[32,44,48]. From these data, which are mainly retrospective or post hoc analyses, it is not possible to conclude whether a lower dose of concurrent IM is equally efficacious and safer in combination therapy. For thiopurines, "therapeutic" 6-TGN levels were required to achieve IFX levels associated with mucosal healing, while a rise in MCV of > 7 fL may be a useful surrogate target if replicated in other studies. For MTX, unlike rheumatologic studies where lower doses appear adequate to maximize anti-TNF levels, in IBD higher doses (15-25 mg weekly) were required to maintain remission. Therefore until well-designed prospective studies prove otherwise, using full doses of IMs as combination therapy appears to be the best option for clinicians.

CAN IMMUNOMODULATORS BE STOPPED AT ANY TIME WHEN USED IN COMBINATION THERAPY?

In combination therapy patients with a high risk of adverse events to continuing therapy and a low risk of disease relapse on treatment withdrawal, cessation of therapy can be considered. Either the anti-TNF or the IM can be stopped, although relapse rates after IM withdrawal are generally lower than relapse rates after anti-TNF discontinuation, making IM withdrawal the more logical strategy[71]. Another rationale for stopping the IM comes from recent data showing that the risk of malignancy with thiopurines, and lymphoma in particular, is associated with the duration of therapy and reduces, or even normalizes, after IMs are ceased. In the CESAME cohort the hazard ratio for lymphoma was 5.28 (95%CI: 2.01-13.9, $P = 0.0007$) for those continuing thiopurines, but became insignificant (HR = 1.02, 95%CI: 2.01-13.9, $P = 0.98$) after they were ceased[41]. More recently in a retrospective cohort study of 36,891 veterans with UC the hazard ratio for developing lymphoma in patients on thiopurines was 4.2 (95%CI: 2.5-6.8, $P < 0.0001$), but reduced to 0.5 (95%CI: 0.2-1.3, $P = 0.17$) after thiopurines were discontinued[42]. In the most-recent meta-analysis combining 18 population-based and referral-centre studies lymphoma risk became significant after 1 year of thiopurine exposure. Amongst population studies standardized incidence ratios (SIR) were increased amongst current (SIR = 5.71, 95%CI: 3.72-10.1), but not former users (SIR = 1.42, 95%CI: 0.86-2.34)[48]. Similar trends of a reduction in malignancy risk after cessation of therapy have been demonstrated in some thiopurine-associated NMSC cohorts[32,72].

The first well-designed, albeit open-label, study of IM withdrawal (the IMID Study) came from the Leuven group in which 80 CD patients in remission on combination therapy for at least 6 mo were randomized to continue or stop IM therapy, with both

groups continuing scheduled maintenance IFX for 2 years. There was no difference in the primary endpoint of patients requiring a decrease in IFX dosing interval (60% in patients continuing IMs *vs* 55% in patients stopping IMs, $P = 0.65$) or stopping IFX (27.5% *vs* 22.5% respectively, P = NS). Mucosal healing rates were also similar between groups. However patients continuing on IMs had significantly higher trough IFX levels (2.87 μg/mL *vs* 1.65 μg/mL, $P < 0.0001$) and correspondingly lower levels of CRP (1.6 mg/L *vs* 2.8 mg/L, $P < 0.005$), suggesting the possibility of differing outcomes between groups over a longer period of follow up[55]. In a single-centre observational study of 48 CD patients on combination therapy for at least 6 mo in whom AZA was stopped, survival without IFX failure was 85% at 12 mo and 41% at 24 mo. Predictors of IFX failure were a duration of combination therapy less than 27 mo (HR = 7.46, 95%CI: 1.64-33.85, $P = 0.01$) and presence of inflammation at the time of IM withdrawal (CRP > 5 mg/L, HR = 4.79, 95%CI: 1.52-15.10, $P = 0.008$, and platelet count > 298 (HR = 4.75, 95%CI: 1.28-17.57, $P = 0.02$)[73]. More recently, in another single-centre, retrospective study the Leuven group assessed the effect of IM withdrawal on IFX trough levels and immunogenicity. Of 158 patients on combination therapy for at least 6 mo (median 13 mo), IM were withdrawn in 117 patients who were followed for a median of 29 mo. Of patients stopping IMs 38% required an increase in IFX dosing interval and 18% stopped IFX. However IFX trough levels were unchanged before and after IM withdrawal (3.2 μg/mL *vs* 3.7 μg/mL respectively, $P = 0.70$). Low IFX trough levels and high CRP at the time of IM withdrawal, and previous IFX dose-escalation prior to IM withdrawal were predictors of subsequent IFX monotherapy failure. Interestingly, no patients with an IFX trough level > 5 μg/mL at the time of IM withdrawal relapsed during the follow up period[74]. From these three studies it can be concluded that the lowest risk of relapse is in patients who are in deep remission (clinical remission and normalized biomarkers including mucosal healing), with good anti-TNF drug levels, after a prolonged period of combination therapy (ideally at least 12 mo) before IMs are withdrawn. Patients with active disease who withdraw IM are more likely to flare and subsequently require optimization of treatment.

Hopefully the upcoming international BIOCYCLE study, which aims to compare outcomes of treatment cycles in patients on combination therapy to outcomes when either the anti-TNF or IM is withdrawn will provide further clarification of the safety of de-escalation strategies in individual patients.

Of relevance to the issue of de-escalation of therapy, two small recent studies have shown that in patients losing response to anti-TNF monotherapy the re- addition of an IM can overcome immunogenicity and recapture response in some patients. In a small series of 5 patients losing response to IFX due to immunogenicity the addition of an IM (thiopurines in 3 patients, MTX in 2 patients) was successful in overcoming ATIs, increasing serum IFX levels and restoring clinical response in all patients[75]. Similar results were demonstrated when thiopurines were added to five patients failing ADA monotherapy, all of whom had previously failed thiopurine monotherapy. Clinical improvement was noted in all patients and repeat endoscopy was performed in four patients, all of whom showed improvement[76].

CONCLUSION

Over the last 15 years there have been great advances in the understanding of the relative roles IMs and anti-TNFs play in the modern management of IBD. It has become recognized that amongst thiopurine naïve patients, combination therapy is more efficacious than monotherapy with either thiopurines or anti-TNF alone, albeit at an increased risk of adverse events, most important of which are infection and malignancy. However questions remain as to how best to position IM use in those who require treatment with an anti-TNF, particularly in IM failures. Many of these are being addressed as we learn more about the pharmacokinetic relationship between anti-TNF and IM use and clinical outcomes. Combination therapy is associated with higher anti-TNF drug levels and less anti-drug antibody production, especially during the first 12 mo. Higher drug levels, in turn, measured post-induction[77-80] and during maintenance therapy[81-84], are associated with favorable clinical outcomes. Whereas it is tempting to equate the beneficial effects of combination therapy solely to an improvement in anti-TNF pharmacokinetics, it must be recognized that this conclusion is at present intuitive rather than evidence based. Prospective studies are needed that assess differences in efficacy, safety and costs between combination therapy *vs* anti-TNF monotherapy with anti-TNF dose-adjustments to achieve similar drug levels[85]. Further research is also needed to determine the effect of varying thiopurine and MTX doses on anti-TNF pharmacokinetics, incorporating both weight-based and metabolite-based (thioguanine nucleotides and MTX polyglutamates[86], for thiopurines and MTX respectively) dose-optimization strategies.

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