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Therapeutic Drug Monitoring for Current and Investigational Inflammatory Bowel Disease Treatments

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Abstract: This article reviews therapeutic drug monitoring (TDM) use for current inflammatory bowel disease (IBD) treatments. IBD comprises Crohn's disease and ulcerative colitis-chronic gastrointestinal inflammatory disorders. Treatment options for moderate to severe IBD include thiopurines; methotrexate; biologic agents targeting tumor necrosis factor, $\alpha_4\beta_7$ integrin or interleukins 12 and 23; and Janus kinase inhibitors. TDM is recommended to guide treatment decisions for some of these agents. Published literature concerning TDM for IBD treatments was reviewed. S.D.L., R.S., and E.V.L. drew on their clinical experiences. Polymorphisms resulting in altered enzymatic activity inactivating thiopurine metabolites can lead to myelotoxicity and hepatotoxicity. Increased elimination of biologic agents can result from immunogenicity or higher disease activity, leading to low drug concentration and consequent nonresponse or loss of response. TDM may aid treatment and dose decisions for individual patients, based on monitoring metabolite levels for thiopurines, or serum drug trough concentration and antidrug antibody levels for biologic agents. Challenges remain around TDM implementation in IBD, including the lack of uniform assay methods and guidance for interpreting results. The Janus kinase inhibitor tofacitinib is not impacted by enzyme polymorphisms or disease activity, and is not expected to stimulate the formation of neutralizing antidrug antibodies. TDM is associated with implementation challenges, despite the recommendation of its use for guiding many IBD treatments. Newer small molecules with less susceptibility to patient variability factors may fulfill the unmet need of treatment options that do not require TDM, although further study is required to confirm this.

Key Words: drug concentrations, ulcerative colitis, Crohn's disease, inflammatory bowel disease, therapeutic drug monitoring

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Inflammatory bowel disease (IBD), which encompasses ulcerative colitis and Crohn's disease, is a chronic, medically incurable disease that can be disabling.[1] A recent systematic review of population-based studies on IBD indicated highest prevalence in Europe (ulcerative colitis: 505 cases/100,000 persons in Norway; Crohn's disease: 322 cases/100,000 persons in Germany) and North America (ulcerative colitis: 286 cases/100,000 persons in the United States; Crohn's disease: 319 cases/100,000 persons in Canada), with the prevalence increasing in newly industrialized countries.[2]

Management of IBD aims to induce and maintain clinical and endoscopic remission.[3,4] Patients with IBD, therefore, require chronic medical treatment that is effective in maintaining remission with acceptable tolerability.[5,6] Treatment options for the maintenance of remission in patients with moderate to severe IBD include, with regional variability: thiopurines [azathioprine (AZA) or 6-mercaptopurine (6-MP)]; methotrexate; biologic agents targeting tumor necrosis factor (TNF; infliximab, adalimumab, certolizumab pegol, or golimumab), $\alpha_4\beta_7$ integrin (vedolizumab) or interleukins 12 and 23 (ustekinumab); and Janus kinase (JAK) inhibitors (tofacitinib).[3,7–10]

This article reviews therapeutic drug monitoring (TDM) use for current IBD treatments. Despite the efficacy of treatments, many patients fail to achieve clinical or endoscopic response (primary nonresponse), or lose response over time (secondary loss of response).[11] One factor that affects the response to thiopurines is variation in the metabolism of the drug among different patients, particularly variations in thiopurine methyltransferase (TPMT) activity.[12] If one's metabolism of thiopurines leads to higher levels of the active 6-thioguanine nucleotide (6-TGN) metabolite, this can not only impact efficacy but can also cause safety issues, as there is a risk of myelotoxicity.[12] In addition, accumulation of the 6-methylmercaptopurine (6-MMP) metabolite can lead to hepatotoxicity.[13] With biologic agents, nonresponse and loss of response can occur due to variation in serum drug exposure at the indicated dose due to factors affecting drug clearance, including patient characteristics, presence of active inflammation, and the formation of antidrug antibodies

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(ADAs)[11,14,15]; however, it is unclear whether trough concentrations (C_{trough}) or area under the curve are more important for achieving a response. Changes to treatment may be warranted to address primary nonresponse or secondary loss of response, with options including, dose-escalation, use of additional immunosuppressant medications, or switching to an alternative treatment.[3,11]

TDM is a clinical decision-making tool that is increasingly being used and recommended to guide and optimize certain treatments for IBD. TDM involves the use of laboratory measurements such as serum drug and ADA concentrations as the basis for dosage adjustments, to reach the drug exposure associated with the highest possible response rate.[3,14,16] Importantly, TDM requires the availability of studies that correlate C_{trough} with clinical effectiveness, so that therapeutic C_{trough} recommendations can be made.[14]

"Proactive" TDM is performed by some providers in patients with clinical or endoscopic response to a given therapy, while the patient is doing well, to optimize treatment and prevent future relapse; in nonresponders or those with loss of response, "reactive" TDM is performed to guide treatment decision-making towards the goal of achieving remission,[16] and to aid understanding of why a patient might have nonresponse or loss of response. The American Gastroenterological Association (AGA) recommends both proactive *TPMT* testing (genotyping or phenotyping) and reactive monitoring of active metabolite levels for patients with active IBD who are receiving thiopurines and suggests reactive TDM in clinical practice for patients with IBD who are receiving anti-TNF agents.[17] The European Crohn's and Colitis Organisation (ECCO) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) also recommend TDM for optimization of treatment outcomes with anti-TNF agents, especially during maintenance.[18] The AGA makes no recommendation regarding proactive TDM for patients with quiescent IBD receiving anti-TNF agents, due to a lack of robust clinical evidence.[17]

Small-molecule treatments with novel modes of action are currently under investigation for IBD, including the sphingosine 1-phosphate receptor modulator, ozanimod, and JAK inhibitors, such as filgotinib and upadacitinib.[19–21] In addition, tofacitinib is an oral, small-molecule JAK inhibitor for the treatment of ulcerative colitis. This article reviews the literature on the use of TDM for thiopurines and biologic agents in the treatment of IBD, as well as data on the pharmacokinetics of tofacitinib in patients with ulcerative colitis, which suggest a lack of need for TDM.

RATIONALE AND RECOMMENDATIONS FOR TDM WITH CURRENT THERAPIES FOR ULCERATIVE COLITIS

Summary

Table 1 shows a summary of important considerations and recommendations for TDM with current therapies for IBD.

Thiopurines

Thiopurines, although not approved for the treatment of IBD by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA),[23,24] have been used in the treatment of IBD for many years.[12] However, up to 28% of patients receiving thiopurines have adverse drug reactions, and around one third of patients discontinue treatment for this reason.[12,25–31] The use of thiopurines in the treatment of IBD can be optimized by TDM, increasing clinical efficacy, and reducing drug-associated toxicity.[32] It is believed that the difference in patients' responses to thiopurines can be partly explained by the variable activity of the enzymes involved in thiopurine metabolism and variable formation of the active metabolites.[12,32] Figure 1 summarizes thiopurine metabolism.

The metabolism of AZA and 6-MP is a complex multienzyme process.[12] If the prodrug AZA is administered, it is first metabolized to 6-MP by glutathione S-transferase.[12,23] There are then 3 main pathways: (1) activation to 6-TGN by hypoxanthine-guanine phosphoribosyltransferase, inosine monophosphate dehydrogenase, and guanosine monophosphate synthetase; (2) inactivation to 6-thiouric acid by xanthine oxidase; and (3) inactivation to 6-MMP or 6-MMP ribonucleotides by TPMT.[12]

Xanthine oxidase is almost absent in erythrocytes, and most likely other hematopoietic tissues, meaning that inactivation in these cells occurs by TPMT methylation only.[23] TPMT activity, therefore, correlates inversely with 6-TGN levels.[23] Although 6-TGN is thought to be the metabolite responsible for therapeutic benefit, its incorporation into DNA[23] and low TPMT activity have been associated with myelosuppression.[12,13,32] The metabolism of thiopurines varies between individuals, due to the involvement of multiple enzymes and, partially, due to genetic variation. Various polymorphisms of the *TPMT* gene have been described, leading to different levels of enzyme activity.[23] TPMT polymorphisms have also been associated with hematotoxicity and, in particular, neutropenia.[13] Furthermore, high TPMT activity, and the consequent accumulation of 6-MMP, causes hepatotoxicity.[13] Genotyping or phenotyping of *TPMT* is therefore recommended[23] before initiation of treatment, to identify patients with intermediate levels of TPMT activity, who may require a dose reduction to minimize the risk of myelotoxicity, and those with low TPMT activity, who are ineligible for treatment. Phenotyping quantifies the biologically active enzyme; the result is, therefore, more representative of the in vivo TPMT activity than that of genotyping,[33] and is preferred by the American College of Gastroenterology.[6]

Recent guidance from the AGA recommends that for adult patients with IBD, in addition to TPMT testing to guide the initial thiopurine dose, thiopurine metabolite levels should be monitored reactively in response to active disease or potential thiopurine toxicity.[17] A target 6-TGN cutoff of 230 to 450 pmol/8×10^8 red blood cells, is recommended when thiopurine is used as monotherapy, although this does not guarantee remission.[17,34] The optimal 6-TGN cutoff when thiopurines are used in combination with anti-TNF agents is uncertain.[17,34] There is a recommendation against routine proactive metabolite monitoring in patients in remission.[17] In addition, ECCO-ESGAR guidelines recommend TDM in IBD patients with loss of response to thiopurines.[18]

While the recommendations for TDM in patients receiving thiopurines have not changed, their long-term use in IBD treatment has recently declined due to the inherent risk of malignancy associated with thiopurine use.[23] Thiopurines can be used in combination with biologic agents since it is thought that the immunosuppressive effect reduces immunogenic potential, reducing the development of ADAs to the biologic, and may also lead to increased trough levels of the biologic agent, which has been associated with higher remission rates.[35,36] Proactive TDM of the thiopurine is often not undertaken if the patient has responded to the combination of biologic and thiopurine; the thiopurine is often discontinued within 1 to 2 years because of the malignancy risk. Furthermore, TDM of the biologic agent can be undertaken to guide dosing in combination therapy.

TABLE 1. Summary of Important Considerations and Recommendations for TDM by Therapy

Therapy	Summary of Important Considerations for TDM	Recommendation of TDM: AGA[17]	ECCO-ESGAR[18]	IBD Sydney Organisation and Australian Inflammatory Bowel Diseases Working Group Consensus Statements[16]	BRIDGe[22]
Thiopurines	Monitoring thiopurine metabolites, particularly 6-MMP and 6-TGN, can optimize treatment with thiopurines Genotype testing, for example, of *TPMT* and *NUDT15*, are also useful to predict thiopurine metabolism	Proactive TPMT testing; reactive metabolite monitoring*	Reactive	NA	NA
Methotrexate	Monitoring liver function and blood counts before and during methotrexate treatment would allow clinicians to reduce dose if necessary, potentially leading to fewer adverse events	NA	NA	NA	NA
Anti-TNFs	TDM of anti-TNF trough levels has been widely adopted in clinical practice to guide dose escalation, introduction of immunosuppressants, or switching to another therapy Monitoring ADA levels is also used to guide management strategies, although clinicians should be aware that ADA levels can vary depending on the type of assay used	Reactive	Reactive	Proactive† and reactive	Proactive and reactive
Vedolizumab	Trough levels of vedolizumab may be useful indicators of efficacy to guide clinicians in dose optimization Further study is required to determine the minimum trough concentration required for clinical remission	NA‡	Recommended when available	NA‡	Reactive
Ustekinumab	Similar to vedolizumab, trough levels of ustekinumab may be useful indicators of efficacy to guide clinicians in dose optimization, although the minimum trough concentration for clinical remission remains to be determined	NA‡	Recommended when available	NA‡	Reactive
Tofacitinib	There is no evidence to suggest that TDM would be of clinical benefit in patients receiving tofacitinib, although this has not been specifically studied	NA	NA	NA	NA

*In patients with active IBD or adverse effects thought to be due to thiopurine toxicity.
†Recommended only when results would change management.
‡Further data required before a recommendation can be made.
ADA indicates antidrug antibody; AGA, American Gastroenterological Association; BRIDGe, Building Research in IBD Globally; ECCO, European Crohn's and Colitis Organisation; ESGAR, European Society of Gastrointestinal and Abdominal Radiology; 6-MMP, 6-methylmercaptopurine; NA, not applicable; NUDT15, nudix hydrolase 15; TDM, therapeutic drug monitoring; 6-TGN, 6-thioguanine nucleotide; TNF, tumor necrosis factor; TPMT, thiopurine methyltransferase.

For example, based on the clinical experiences of S.D.L., R.S., and E.V.L., in a patient receiving infliximab and AZA combination therapy, AZA could be stopped if the trough infliximab concentration is ~10 μg/mL after at least 12 months of treatment. However, if the trough infliximab concentration is in the region of 4 μg/mL, it may be beneficial to increase the infliximab dose before stopping AZA.

In patients who have not achieved remission with combination biologic and thiopurine therapy, one could optimize the dosing of the thiopurine via TDM. However, there are no studies to validate whether this impacts disease activity. In addition, if the long-term goal is to discontinue from thiopurine use, the utility of this modification is not clear, and the authors would usually consider a therapy change rather than a dose change. In patients who have achieved remission on combination biologic and thiopurine therapy, a dose reduction of thiopurine is feasible and results in similar outcomes to the "therapeutic" dose of thiopurines.[37] However, there is no evidence that this reduction lowers long-term malignancy risk. In contrast, in patients who have achieved remission on combination biologic and thiopurine therapy, but with low-level ADAs, the authors would consider TDM to assess whether the thiopurine dose can be increased to prevent further ADA formation.

Although there is a known association between *TPMT* polymorphisms and thiopurine-associated myelosuppression (and its manifestation as leukopenia), only around one quarter of

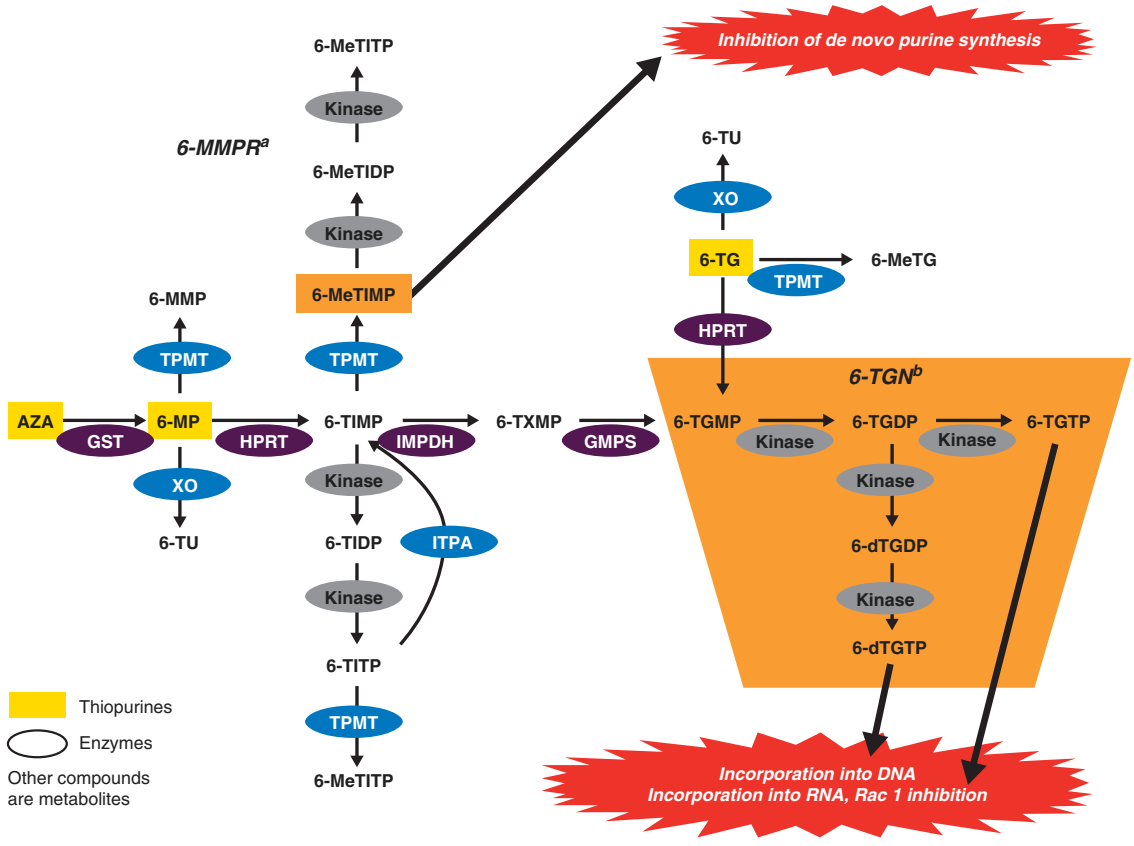

FIGURE 1. Thiopurine metabolism.[12] [a]The metabolites 6-MeTIMP, 6-MeTIDP, and 6-MeTITP together form 6-MMPR. [b]6-TGMP, 6-TGDP, and 6-TGTP together form active metabolites 6-TGN. The orange box indicates the production of active metabolites during thiopurine metabolism. AZA indicates azathioprine; 6-dTGDP, deoxy-6-thioguanine diphosphate; 6-dTGTP, deoxy-6-thioguanine triphosphate; GMPS, guanosine monophosphate synthetase; GST, glutathione S-transferase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; IMPDH, inosine monophosphate dehydrogenase; ITPA, inosine triphosphatase; 6-MeTG, 6-methylthioguanine; 6-MeTIDP, 6-methylthioinosine diphosphate; 6-MeTIMP, 6-methylthioinosine monophosphate; 6-MeTITP, 6-methylthioinosine triphosphate; 6-MMP, 6-methylmercaptopurine; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 6-MP, 6-mercaptopurine; 6-TGDP, 6-thioguanine diphosphate; TG, thioguanine; 6-TGMP, 6-thioguanine monophosphate; 6-TGN, 6-thioguanine nucleotide; 6-TGTP, 6-thioguanine triphosphate; 6-TIDP, 6-thioinosine diphosphate; 6-TIMP, 6-thioinosine monophosphate; TPMT, thiopurine methyltransferase; 6-TITP, 6-thioinosine triphosphate; 6-TU, 6-thiouric acid; 6-TXMP, 6-thioxanthosine monophosphate; XO, xanthine oxidase.

patients with IBD and thiopurine-associated myelosuppression/leukopenia carry a *TPMT* mutation.[38] Furthermore, *TPMT* mutations are less common in Asian populations than in European populations, but thiopurine-induced leukopenia is more common in Asian patients than in European patients.[39] A nudix hydrolase (*NUDT*) *15* variant allele has been strongly associated with thiopurine-induced leukopenia in Korean and Chinese patients with Crohn's disease.[39,40] In addition, 3 *NUDT15* variants have been associated with thiopurine intolerance in Asian and non-Asian patients with acute lymphoblastic leukemia.[41] It has been suggested that *NUDT15* genotyping may become a requirement for patients initiating thiopurine therapy,[42] and there are now laboratories that offer clinical *NUDT15* genotype testing.

Methotrexate

Methotrexate is an antimetabolite that is antiproliferative and immunosuppressive: it impairs DNA synthesis (via inhibition of dihydrofolate reductase), decreases the production of proinflammatory cytokines and induces lymphocyte apoptosis.[43,44] Methotrexate is mainly used to treat Crohn's disease; the use of methotrexate in patients with ulcerative colitis is controversial.[44] A recent study of 179 patients with active ulcerative colitis found that 91 patients (51%) achieved steroid-free response with methotrexate induction therapy; however, in the 84 patients who then received maintenance therapy, methotrexate was not superior to placebo in the prevention of relapse.[45] Adverse events in patients receiving methotrexate treatment include myelosuppression and hepatotoxicity, both of which are dose-dependent.[44]

Despite this, there is a lack of data available evaluating methotrexate therapy in IBD, and there are no societal recommendations for monitoring hepatotoxicity.[13] The authors recommend complete blood count and liver function tests before, and 1 month after, initiation of methotrexate. If liver function test results are elevated, then the authors recommend reducing the methotrexate dose. In patients with normal test results remaining on methotrexate, the authors recommend repeating these tests every 2 to 4 months. Simultaneous treatment with folic acid can reduce the adverse events associated with methotrexate treatment,[44] and is advisable.[7] Several polymorphisms in enzymes involved in the metabolism of folic acid are associated with the toxicity of methotrexate, though study results are conflicting.[13]

TABLE 2. ADA Formation Rates for Biologic Agents in Patients With IBD

	Frequency of ADA Formation Range of Reported % (Number of Studies)			
	Crohn's Disease		Ulcerative Colitis	
Drug	Strand et al[58]	Vermeire et al[59]	Strand et al[58]	Vermeire et al[59]
Infliximab	3-83 (29)	3-61 (22)	6-46 (10)	6-41 (8)
Adalimumab	0-35 (13)	0-35 (11)	3-5 (3)	3-5 (3)
Golimumab	—	—	0-19 (8)	0-3 (2)
Certolizumab pegol	3-25 (6)	3-25 (4)	—	—
Ustekinumab	0-1 (2)	1 (1)	—	—
Vedolizumab	*	1-4 (2)	*	4 (1)

*Not included in the analysis.
— indicates no publications available; ADA, antidrug antibody; IBD, inflammatory bowel disease.

Biologic Agents

Anti-TNF Agents

The introduction of biologic agents has greatly improved the management of IBD.[46] However, primary nonresponse and secondary loss of response are significant clinical problems, particularly with anti-TNF agents.[15,47] In 2011, Ben-Horin and Chowers[47] reviewed the literature on the loss of response to anti-TNF agents in Crohn's disease. They found that 23% to 46% of patients who initially responded to anti-TNF agents had experienced the secondary loss of response based on the need for dose intensification by 12 months of therapy.[47] Several factors can cause nonresponse or loss of response, including patient characteristics such as increased body mass index,[48] female sex,[49] increased age,[50] and severely active IBD leading to high inflammatory burden and fecal loss of the drug.[15] Parenteral administration of large molecule biologic agents can lead to the development of ADAs (immunogenicity) that reduce serum active-drug concentrations, and this is the main cause of loss of response to anti-TNF agents.[51–54] In addition to the use of thiopurines in combination with biologic agents to reduce immunogenicity, other immunosuppressants, mainly methotrexate, can be of similar use.[54]

Reported rates of ADAs vary across studies of IBD and may depend on the assay used. Enzyme-linked immunosorbent assays (ELISA) are frequently used because they are simple and inexpensive. However, they can be subject to drug interference, in which the circulating drug can inhibit the capture and detection of ADA, even at relatively low drug concentrations.[55–57] Drug-tolerant assays, such as the radioimmunoassay and homogenous mobility shift assay, demonstrate reduced cross-reactivity compared with ELISAs, allowing ADA to be measured when the drug is present.[56] However, compared with ELISA, radioimmunoassay is not as simple to use, and homogenous mobility shift assay has a higher cost.[57] Table 2 summarizes ADA formation rates for biologic agents in patients with Crohn's disease and ulcerative colitis, reported in 2 recent systematic reviews of the immunogenicity of biologic agents.[58,59]

Evidence from clinical studies of patients with IBD has shown that serum C_{trough} of anti-TNF agents and ADA concentrations are associated with clinical and endoscopic outcomes.[11,60–66] Reactive monitoring of anti-TNF drug concentrations to inform decisions about management strategies (eg, dose-escalation, use of combination therapy with immunosuppressants, or switching to another therapy) has been widely adopted in clinical practice.[11,17] Figure 2 shows a summary of guidance in the literature for TDM of anti-TNF agents for IBD.[14,16,46,54,67] To measure C_{trough} values, blood samples are collected just before the next administration of the biologic agent.[14] For adalimumab, however, it has been suggested that blood samples can be collected at any time point in the treatment cycle due to its uniform concentration—time profile.[68] However, a subsequent study found that adalimumab concentrations are only uniform for the first 9 days of the treatment cycle, and it is best to perform TDM at the trough for adalimumab.[69] In contrast, one study found that adalimumab concentrations

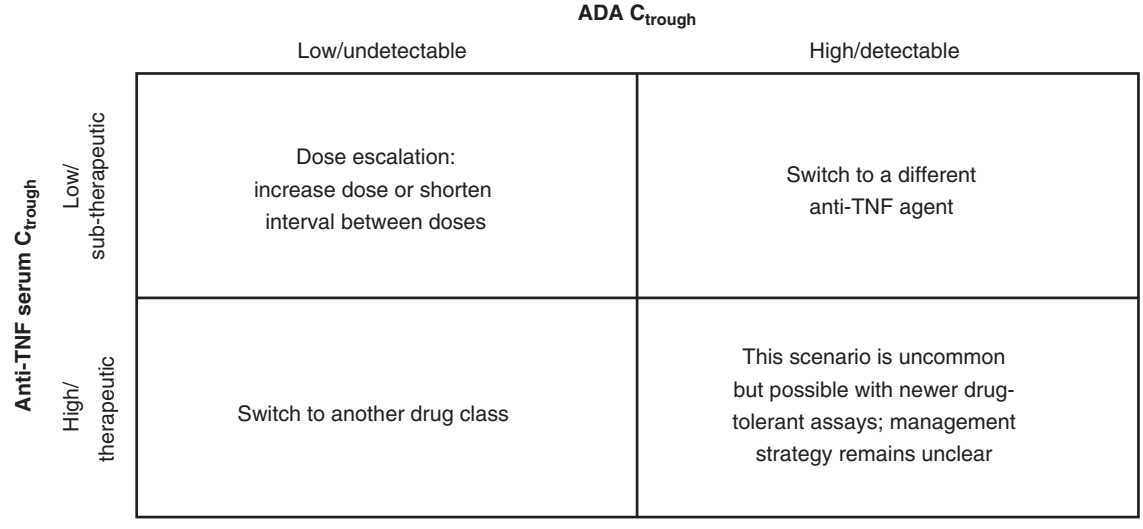

FIGURE 2. Summary of guidance for TDM of anti-TNF agents. This summary assumes that TDM is reactive and that there is objective evidence of inflammation present. ADA indicates antidrug antibody; C_{trough}, trough concentration; TDM, therapeutic drug monitoring; TNF, tumor necrosis factor.

were not correlated with remission, suggesting that TDM would not be useful or informative in patients receiving adalimumab.[70] In addition to reactive TDM, the IBD Sydney Organisation and Australian Inflammatory Bowel Diseases Consensus Working Group and Building Research in IBD Globally (BRIDGe) recommend proactive TDM of anti-TNF agents after induction and at least once in maintenance in patients with IBD.[16,22]

Recent guidance from the AGA suggests that for adults receiving maintenance anti-TNF treatment for active IBD, reactive TDM for drug C_{trough} should be conducted to guide changes in treatment but recommendations on proactive TDM or monitoring during induction therapy cannot currently be made.[17] Similarly, ECCO-ESGAR guidelines suggest that measuring trough levels can guide treatment decisions in IBD patients with secondary loss of response to anti-TNF treatments.[18] However, there are no specific trough level recommendations currently available within these guidelines. Target C_{trough} recommended by the AGA for reactive TDM in adults receiving maintenance anti-TNF treatment for active IBD are shown in Table 3.[17] The AGA-suggested target C_{trough} were derived from a technical review of cross-sectional studies of patients in various stages of clinical response or remission receiving maintenance treatment.[71] Because of insufficient data at the time of publication, this AGA guidance does not cover TDM in patients treated with vedolizumab or ustekinumab.[17]

It is important to note that although the AGA have made these recommendations about therapeutic biologic C_{trough}, other recommendations vary[46]; for example, the following C_{trough} have been recommended in the published literature: >2 μg/mL[72]; >3 μg/mL[73]; 3 to 7 μg/mL[74]; and 5 to 10 μg/mL[75] for infliximab; and ≥ 8 μg/mL for adalimumab.[76] Because TDM-recommended C_{trough} is based on associations, achieving a target C_{trough} does not guarantee a response. A proportion of patients with "therapeutic" C_{trough} will be nonresponders; likewise, a proportion of patients with "subtherapeutic" C_{trough} will be responders. Therefore, rather than using TDM to target a particular drug level, the true target should arguably be achieving endoscopic remission, for which individual C_{trough} will vary. As with clinical symptoms, mucosal healing has been associated with TDM[77]; however, there are currently no definitive studies to guide practitioners regarding target C_{trough} for achieving endoscopic remission.

Vedolizumab

Vedolizumab targets $\alpha_4\beta_7$ integrin rather than TNF.[78] In the GEMINI 1 and GEMINI 2 studies, ADAs were detected in 56 of 1434 (4%) patients treated with vedolizumab continuously for up to 52 weeks; and were persistent (in 2 or more consecutive samples) in only 9 of these 56 patients.[78] Patients with ADA persistence generally had lower serum drug C_{trough} compared with the general study population[79]: 6 patients had undetectable vedolizumab concentrations, 2 had reduced vedolizumab concentrations, and data were not available for the ninth patient.[80] None of these 9 patients achieved clinical remission at weeks 6 or 52 of the studies.[80] More recently, in a prospective study of the formation of vedolizumab ADAs in 106 patients with IBD, ADAs were detected in 17% of patients in induction therapy and 3% of patients in maintenance therapy.[81] By contrast, in another study, serum samples of 179 vedolizumab-treated patients with IBD were tested using a drug-resistant assay for ADA detection.[82] Of the 179 patients, 4 (2.2%) were ADA-positive, but the ADAs were all transient.[82] Further research is required to fully assess the immunogenicity of vedolizumab.

Compared with anti-TNF agents, there are substantially less data to inform guidelines on TDM for vedolizumab.[17] However, a recent study investigated whether serum vedolizumab C_{trough} during induction treatment can determine whether patients will require additional doses within the first 6 months.[83] At week 6, C_{trough} <18.5 μg/mL were associated with the need for extended therapy within the first 6 months, and all patients with C_{trough} <19.0 μg/mL at week 6 regained a secondary response after drug optimization at week 10.[83] The same research group then investigated the relationship between vedolizumab C_{trough} and mucosal healing in patients with IBD in their first year of vedolizumab treatment; C_{trough} ≥ 18.0 μg/mL at week 6 was associated with mucosal healing.[84] These findings indicate that vedolizumab C_{trough} monitoring may help clinicians in drug optimization decision-making.[83] In a cross-sectional study of 171 patients with IBD treated with vedolizumab, C_{trough} was significantly higher among patients with Crohn's disease with normal C-reactive protein than those with elevated C-reactive protein; however, no difference was seen in patients with ulcerative colitis, and there was no significant difference in C_{trough} between patients with IBD with and without mucosal healing.[85] Currently, there is evidence of C_{trough} that are associated with mucosal healing and clinical remission,[84,86] but there are no definitive studies to guide practitioners regarding the target C_{trough} for achieving endoscopic remission. Further studies are required to elucidate the role of TDM in vedolizumab treatment.

Ustekinumab

Ustekinumab is an inhibitor of the p40 subunit of interleukin 12 and interleukin 23 and is an effective treatment for patients with Crohn's disease.[87] The association between C_{trough} of ustekinumab and endoscopic outcome in clinical practice has been investigated in patients with Crohn's disease who were refractory or intolerant to anti-TNF agents.[88] Patients received ustekinumab 90 mg subcutaneously at weeks 0, 1, and 2 during induction and every 4 or 8 weeks during maintenance.[88] The endoscopic outcome was assessed at week 10 postinduction and at week 26 or later during maintenance; the results demonstrated that a greater proportion of patients with C_{trough} of ustekinumab >4.5 μg/mL (75.9%) had an endoscopic response compared with those with C_{trough} <4.5 μg/mL.[88] Patients with an ustekinumab C_{trough} of >4.5 μg/mL by at least week 26 also had a lower mean level of C-reactive protein. The authors concluded from this study that maintenance C_{trough} of ustekinumab >4.5 μg/mL at 26 weeks or later was associated with biomarker reduction and endoscopic response.[88] The majority (77.4%) of patients were on a maintenance dose of ustekinumab 90 mg every 4 weeks subcutaneously,[88] which, to our knowledge, has not otherwise been studied, and is higher

TABLE 3. AGA-suggested Target C_{trough} for Reactive TDM in Patients With Active IBD on Anti-TNF Maintenance Therapy[17]

Drug	Suggested Target C_{trough} (μg/mL)
Infliximab	≥ 5
Adalimumab	≥ 7.5
Golimumab	Unknown
Certolizumab pegol	≥ 20

AGA indicates American Gastroenterological Association; C_{trough}, trough concentration; IBD, inflammatory bowel disease; TDM, therapeutic drug monitoring; TNF, tumor necrosis factor.

than the maintenance dose studied in the pivotal trials.[87,89] A more recent report analyzed data on ustekinumab serum concentrations from induction studies of patients with moderate to severe Crohn's disease treated with ustekinumab for 8 weeks following a single intravenous dose, and from a maintenance study of patients with a response in the induction study who then received subcutaneous injections of ustekinumab every 8 or 12 weeks for 44 weeks.[90] Ustekinumab serum concentrations were associated with rates of clinical remission and endoscopic endpoints; ustekinumab C_{trough} of >0.8 μg/mL was associated with maintenance of clinical remission in a higher proportion of patients versus C_{trough} ≤ 0.8 μg/mL.[90] There is therefore still some uncertainty as to the exact cutoff value for minimum C_{trough} of ustekinumab.

CHALLENGES IN IMPLEMENTING TDM IN REAL-WORLD PRACTICE

The benefit of TDM for thiopurine treatment of IBD is unclear. TPMT testing can identify patients with low or absent enzyme activity[23]; it, therefore, has the potential to avoid myelosuppression. Unfortunately, the TPMT assay is not routinely performed in many clinics. Furthermore, most thiopurine-related hematologic adverse events occur despite normal TPMT status; TPMT testing therefore cannot substitute routine complete blood count monitoring in patients receiving thiopurines.[23,38,71] TPMT testing does not impact the burden of the routine monitoring that is mandatory for all thiopurine-treated patients, regardless of TPMT status.[23]

Thiopurine metabolite monitoring can identify those patients who preferentially convert 6-MP to 6-MMP and fail to achieve sufficient 6-TGN levels, thus having the potential for accumulation of 6-MMP and hepatotoxicity.[91] Therefore, metabolite monitoring is of particular therapeutic benefit in cases where 6-TGN levels are very low and 6-MMP are very high, as it allows clinicians to avoid unnecessary thiopurine dose escalation that may lead to 6-MMP-induced hepatotoxicity.[34,92] However, to date, no randomized controlled trials have demonstrated the benefit of thiopurine treatment decisions based on 6-TGN concentrations. In a prospective randomized controlled trial in patients with Crohn's disease, standard AZA dosing was compared with AZA dosing adapted to maintain 6-TGN concentrations of 250 to 400 pmol/8×10^8 erythrocytes; the 2 dosing strategies led to the same 6-TGN concentrations and remission rates.[93] In another randomized controlled trial in patients with Crohn's disease treated with AZA, standard dosing was compared with dosing that was individualized by baseline TPMT activity and target 6-TGN concentrations.[94] Although there was a trend favouring individualized dosing, the 2 dosing approaches did not differ in efficacy to a statistically significant extent.[94]

Although a target 6-TGN cutoff of 230 to 450 pmol/8×10^8 red blood cells is recommended when thiopurine is used as monotherapy, there is no clear consensus on target 6-TGN levels when thiopurines are used in combination with anti-TNF agents.[17,34] In a cross-sectional study of patients with IBD treated with infliximab and a thiopurine, a 6-TGN level cutoff of >125 pmol/8×10^8 red blood cells was the best predictor of higher infliximab levels.[95] Targeting lower 6-TGN levels that are associated with therapeutic levels of infliximab may minimize toxicity in patients receiving combination thiopurine and anti-TNF therapy,[95] although further guidance is required.

Despite the AGA guidance and available clinical evidence for TDM with biologic agents, key uncertainties remain. For example, although target C_{trough} have been established for infliximab, adalimumab, and certolizumab pegol maintenance treatment, the data for infliximab are more robust than those for adalimumab. There is also insufficient evidence to establish target cutoffs for golimumab, vedolizumab, and ustekinumab, and optimal C_{trough} for induction therapy are uncertain.[17]

In addition, target C_{trough} must not be treated as absolute values, as they may vary based on several factors including target endpoint (clinical remission vs. mucosal healing); disease severity (high activity vs. low activity); or phase of therapy (induction vs. maintenance).[71] Furthermore, a small proportion of patients may not be in remission at the recommended target C_{trough}, and targeting a higher C_{trough} may sometimes be beneficial in these cases.[71] Therefore, the application of target C_{trough} in practice is not straightforward.

Another challenge in implementing TDM in IBD is that there is a lack of guidance for interpretation of results and a lack of standardization among commercially available assays.[67,96] Factors such as drug interference can influence the measurement of serum concentrations of biologic agents and ADA levels, which can result in poor specificity, sensitivity, and reproducibility; for example, a commercially available ELISA detected false-positive infliximab levels in 18% of tested samples.[91] Furthermore, there is uncertainty in the cutoffs for high-titer and low-titer ADA levels measured with available assays. At a sufficiently high-titer, there may be limited benefit in dose escalation, and switching to a different agent in the same drug class may be of greater benefit.[17] This lack of consensus could delay some patients receiving concomitant immunosuppressants, which have been shown to restore serum concentrations of infliximab and clinical response in patients with IBD.[97] Further studies are required to derive optimal low-titer and high-titer ADA cutoff levels.

Several randomized clinical trials have investigated the effect of TDM on clinical outcomes with TDM versus clinical symptom-based therapy modification. The Trough Concentration Adapted Infliximab Treatment (TAXIT) randomized controlled trial compared concentration-based dosing (by TDM) to a 3 to 7 μg/mL target C_{trough} with clinical symptom-based therapy in patients with IBD who were responding to infliximab maintenance therapy.[98] Although TDM resulted in more efficient use of the drug during the optimization phase of the study, continued concentration-based dosing was not superior to clinically based dosing for achieving remission after 1 year.[98] However, there is some controversy regarding how the TAXIT study was designed and performed. The Tailored Treatment with Infliximab for Active Crohn's Disease (TAILORIX) randomized controlled trial compared proactive C_{trough}-based dose intensification with symptom-based intensification in patients receiving infliximab for Crohn's disease.[99] Dose intensification based on symptoms, biomarkers, and infliximab C_{trough} was not superior to that based on symptoms alone in achieving sustained corticosteroid-free clinical remission.[99] Further studies into proactive TDM in IBD treatment are required to ascertain its significance.

While there are challenges in the implementation of TDM, there are also burdens on the patient. The need to measure C_{trough} requires assessment immediately before dosing, which means that a patient with nonresponse or loss of response may have to wait until the next treatment for dose escalation with a reactive TDM approach. The patient

also has to undergo additional blood testing and follow-up. In addition to the practical burden, there may also be a financial burden; while TDM may benefit some patients, the costs are not yet well-defined and may exceed or be less than the costs without TDM. A recent meta-analysis of 9 studies of infliximab maintenance therapy in adults with IBD, found that although TDM was not associated with superior clinical remission rates, there was a cost-benefit.[100] Further research is needed to assess the costs of TDM for drugs other than infliximab.

APPROVED AND INVESTIGATIONAL SMALL-MOLECULE TREATMENTS

Available small-molecule treatments for IBD, or those under investigation for IBD, are unlikely to induce the formation of neutralizing ADAs, in contrast with biologic agents.[101] In addition, as these drugs are not used in combination with thiopurines, TPMT, and metabolite monitoring are not necessary, and the toxicity concerns related to thiopurine use are not present. Therefore, TDM is not likely to be necessary to optimize the treatment of IBD with small-molecule therapies. However, it should be noted that other concerns have been associated with these treatments.

Sphingosine 1-Phosphate Receptor Modulators

The sphingosine 1-phosphate receptor modulator, ozanimod, induces peripheral lymphocyte sequestration, which may reduce the level of activated lymphocytes within the gastrointestinal tract, and has been investigated in a phase 2 study in patients with ulcerative colitis.[19] The pharmacokinetics of ozanimod are linear, dose-proportional, and subject to low to moderate intersubject variability with a high steady-state volume of distribution, moderate apparent oral clearance, and an estimated elimination half-life of 20 hours.[19] Following the cessation of treatment with ozanimod, lymphocyte counts have been shown to recover to normal range within 3 days—a consequence of the short half-life of ozanimod—offering flexibility to change treatment or treat opportunistic infections.[102] However, a plateau in the lymphocyte count reduction has been observed at doses of 1 to 1.5 mg.[103]

JAK Inhibitors

Filgotinib is a JAK inhibitor that is being investigated as a treatment for moderate to severe Crohn's disease.[20] In the randomized controlled FITZROY study, at week 10, filgotinib induced clinical remission in significantly more patients than placebo.[20] Pharmacokinetic and pharmacodynamic studies of filgotinib have demonstrated rapid absorption in healthy volunteers, with a half-life of 6 hours.[104] Filgotinib is metabolized, losing the cyclopropyl carboxylic acid group to form an active metabolite with a similar JAK1 selectivity profile as the parent compound.[104] The filgotinib metabolite was shown to reach a maximum plasma concentration within 3 to 5 hours followed by a slow decrease, with a half-life of 23 hours.[104] Therefore, the times to peak and decline in the plasma level of the metabolite are longer than the drug's, and the rate-limiting step may be the elimination of, as opposed to the formation of, the metabolite.[104] The long duration of JAK1 inhibition following filgotinib dosing suggests that the formation of a "major metabolite" contributes to the overall pharmacodynamics of filgotinib treatment.[104]

Upadacitinib is another JAK inhibitor under investigation as a treatment for moderate to severe Crohn's disease.[21] In the randomized controlled dose-ranging CELEST study, upadacitinib at doses of 6 mg twice daily (bid) and higher, demonstrated endoscopic improvement and clinical benefit in patients with moderate to severe Crohn's disease.[21] Although the pharmacokinetics of upadacitinib have not yet been reported in patients with Crohn's disease, there are reports in healthy volunteers and patients with rheumatoid arthritis (RA). Upadacitinib has been shown to follow dose-proportional biexponential disposition, with a terminal elimination half-life of 6 to 16 hours and a functional half-life of 3 to 4 hours.[105] Oral clearance, steady-state volume of distribution, absorption lag time and mean absorption time have been estimated (95% bootstrap confidence interval) as 39.7 (37.8 to 41.5) L/hour, 210 (196 to 231) L, 0.48 (0.47 to 0.49) hours, and 0.08 (0.04 to 0.12) hours, respectively, in a typical healthy male.[106] In patients with RA, sex, renal impairment, and body weight did not show clinically relevant effects on upadacitinib pharmacokinetics.[106]

Tofacitinib is an oral, small-molecule JAK inhibitor for the treatment of ulcerative colitis. In phase 3 OCTAVE trials, tofacitinib demonstrated efficacy in the induction and maintenance of remission in patients with moderate to severe ulcerative colitis.[107]

Tofacitinib: Pharmacokinetic Considerations With Respect to TDM

Pharmacokinetic analyses have shown that tofacitinib is rapidly absorbed.[108] The primary clearance mechanisms are renal (around 30%) and hepatic (around 70%), with most metabolism attributable to cytochrome P450 (CYP) 3A4 and a minor contribution from CYP2C19.[108] Polymorphisms of CYP3A4 in different populations are not associated with low hepatic CYP3A4 protein expression or low CYP3A4 activity[109]; such polymorphisms are therefore not of concern regarding drug metabolism. An exploratory evaluation that genotyped patients for polymorphisms of the *CYP2C19* gene suggested that these polymorphisms do not affect tofacitinib pharmacokinetics,[110] which contrasts with the effect of polymorphisms on thiopurine metabolism and the consequent risk of myelotoxicity.[23]

In the OCTAVE trials, there was no decrease in plasma concentrations of tofacitinib in individual patients with ulcerative colitis with either the 5 mg bid or 10 mg bid doses during treatment (up to 52 wk), as shown in Figure 3.[111] Baseline disease activity (albumin levels, Mayo score) was not significantly associated with plasma concentrations of tofacitinib during induction or maintenance,[107] indicating that tofacitinib concentration is not expected to be lower in patients with the highest disease activity, in contrast to biologic agents.[112] Tofacitinib plasma concentration is not a meaningful determinant of efficacy, and no loss of efficacy due to low plasma concentration was identified in clinical trials.[107,111]

The pharmacokinetic profile of tofacitinib in patients with ulcerative colitis is consistent with that of other conditions, such as RA, indicating that plasma concentrations of tofacitinib are unaffected by colonic inflammation in ulcerative colitis,[10,107,108,113] in contrast to biologic agents.[112] In addition, there is no clinically relevant effect of intrinsic patient characteristics (age, weight, gender, and race) on tofacitinib exposure, meaning that no dose adjustment is required to account for differences in these characteristics among patients[9]; these results support that there is a lack of clinically relevant polymorphisms in tofacitinib metabolic pathways. While the utility of TDM for tofacitinib treatment

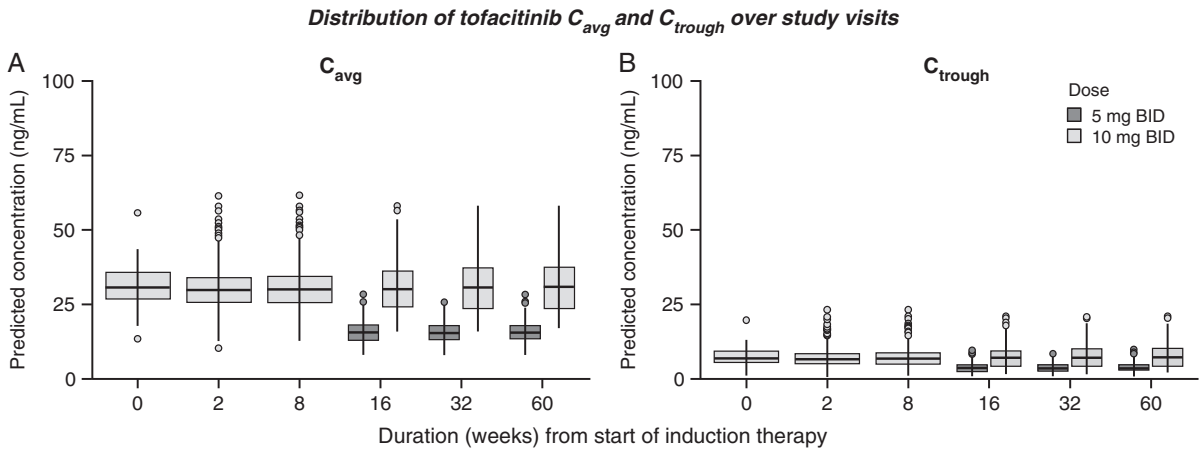

FIGURE 3. Distribution of tofacitinib average (A) and trough (B) plasma concentration from the start of induction therapy to week 52 of maintenance therapy (total weeks: 60) with tofacitinib 5 mg bid (dark) and tofacitinib 10 mg bid (light). bid indicates twice daily; C_{avg}, average concentration; C_{trough}, trough concentration.

has not been specifically studied, these findings suggest it is unlikely that TDM would be of any clinical value during tofacitinib therapy.

CONCLUSIONS

Nonresponse or loss of response to available therapies for IBD occurs in many patients. TDM is increasingly being used by gastroenterologists treating patients with IBD to inform decisions about changes to treatment required due to nonresponse or loss of response. For thiopurines, differences in patients' responses can be accounted for by variation in their levels of TPMT, an enzyme involved in the metabolism of the drug. In addition, low levels of TPMT can lead to high levels of the active metabolite 6-TGN, which are associated with myelotoxicity. Polymorphisms within the gene encoding TPMT can confer low levels of TPMT activity. TDM for thiopurines, therefore, includes genotyping or phenotyping to identify patients with intermediate levels of TPMT activity who may require a dose reduction to minimize the risk of myelotoxicity and to find those patients with low TPMT activity who are ineligible for treatment.

For biologic agents, nonresponse and loss of response can occur due to reductions in drug C_{trough}, which is often because of the presence of ADAs. TDM for biologic agents, therefore, involves monitoring of drug C_{trough} and ADA levels. However, despite the availability of official recommendations on TDM, there is still a lack of consensus on how and when it should be implemented in practice, and what impact it has on clinical outcomes. In addition, there is a lack of standardized assays for monitoring ADA levels. Together, these findings suggest that further guidance on implementing TDM is required to fully optimize the treatment of IBD with biologic agents.

Alternatively, therapies that avoid the need for TDM have the potential to reduce extensive monitoring. Orally administered small-molecule treatments may not be susceptible to neutralizing immunogenicity or variable metabolism. In phase 3 studies of the oral, small-molecule JAK inhibitor tofacitinib, plasma concentrations at any given dose across a treatment group were not reduced during 1-year maintenance therapy and were not affected by disease activity. TDM is therefore unlikely to provide any incremental benefit in the treatment of ulcerative colitis with tofacitinib. However, TDM remains a key recommendation for many IBD treatments.

REFERENCES

1. Ordás I, Eckmann L, Talamini M, et al. Ulcerative colitis. *Lancet*. 2012;380:1606–1619.
2. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017; 390:2769–2778.
3. Harbord M, Eliakim R, Bettenworth D, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. part 2: current management. *J Crohns Colitis*. 2017;11:769–784.
4. Katz JA. Management of inflammatory bowel disease in adults. *J Dig Dis*. 2007;8:65–71.
5. Bressler B, Marshall JK, Bernstein CN, et al. Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the Toronto consensus. *Gastroenterology*. 2015;148: 1035–1058.
6. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol*. 2010;105: 501–523.
7. Gomollón F, Dignass A, Annese V, et al. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis*. 2017;11:3–25.
8. Terdiman JP, Gruss CB, Heidelbaugh JJ, et al. American Gastroenterological Association Institute guideline on the use of thiopurines, methotrexate, and anti-TNF-α biologic drugs for the induction and maintenance of remission in inflammatory Crohn's disease. *Gastroenterology*. 2013;145:1459–1463.
9. European Medicines Agency. Xeljanz (tofacitinib citrate)—summary of product characteristics; 2018. Available at: www.medicines.org.uk/emc/medicine/33167. Accessed September 2, 2019.
10. US Food and Drug Administration. Xeljanz® highlights of prescribing information; 2018. Available at: http://labeling.pfizer.com/showlabeling.aspx?id=959. Accessed December 4, 2018.
11. Kopylov U, Ben-Horin S, Seidman E. Therapeutic drug monitoring in inflammatory bowel disease. *Ann Gastroenterol*. 2014;27:304–312.

12. Moon W, Loftus EV Jr. Review article: recent advances in pharmacogenetics and pharmacokinetics for safe and effective thiopurine therapy in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2016;43:863–883.
13. Tran-Minh ML, Sousa P, Maillet M, et al. Hepatic complications induced by immunosuppressants and biologics in inflammatory bowel disease. *World J Hepatol*. 2017;9:613–626.
14. Dreesen E, Bossuyt P, Mulleman D, et al. Practical recommendations for the use of therapeutic drug monitoring of biopharmaceuticals in inflammatory diseases. *Clin Pharmacol*. 2017;9: 101–111.
15. Brandse JF, van den Brink GR, Wildenberg ME, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. *Gastroenterology*. 2015;149:350–355.
16. Mitrev N, Vande Casteele N, Seow CH, et al. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther*. 2017;46:1037–1053.
17. Feuerstein JD, Nguyen GC, Kupfer SS, et al. American Gastroenterological Association Institute Guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology*. 2017;153:827–834.
18. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. 2019;13:144–164.
19. Sandborn WJ, Feagan BG, Wolf DC, et al. Ozanimod induction and maintenance treatment for ulcerative colitis. *N Engl J Med*. 2016;374:1754–1762.
20. Vermeire S, Schreiber S, Petryka R, et al. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the FITZROY study): results from a phase 2, double-blind, randomised, placebo-controlled trial. *Lancet*. 2017;389:266–275.
21. Sandborn WJ, Feagan B, Panes J, et al. Safety and efficacy of upadacitinib (ABT-494), an oral JAK1 inhibitor, as induction therapy in patients with Crohn's disease: results from CELEST. *United European Gastroenterol J*. 2017;5(suppl 1): A3–A4; OP007.
22. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2019;17:1655–1668.
23. US Food and Drug Administration. IMURAN® (azathioprine) product information; 2011. Available at: www.accessdata.fda.gov/drugsatfda_docs/label/2011/016324s034s035lbl.pdf. Accessed September 2, 2019.
24. European Medicines Agency. EPAR summary for the public: Xaluprine; 2012. Available at: www.ema.europa.eu/documents/overview/xaluprine-epar-summary-public_en.pdf. Accessed September 2, 2019.
25. Schwab M, Schäffeler E, Marx C, et al. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. *Pharmacogenetics*. 2002;12:429–436.
26. Ansari A, Hassan C, Duley J, et al. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2002;16:1743–1750.
27. Marinaki AM, Ansari A, Duley JA, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics*. 2004;14:181–187.
28. Chaparro M, Ordás I, Cabré E, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis*. 2013;19: 1404–1410.
29. Ansari A, Arenas M, Greenfield SM, et al. Prospective evaluation of the pharmacogenetics of azathioprine in the treatment of inflammatory bowel disease. *Aliment Pharmacol Ther*. 2008;28:973–983.
30. Jharap B, Seinen ML, de Boer NK, et al. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis*. 2010;16:1541–1549.
31. Seinen ML, van Asseldonk DP, Mulder CJ, et al. Dosing 6-thioguanine in inflammatory bowel disease: expert-based guidelines for daily practice. *J Gastrointestin Liver Dis*. 2010;19: 291–294.
32. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology*. 2000;118: 705–713.
33. Ford L, Graham V, Berg J. Whole-blood thiopurine S-methyltransferase activity with genotype concordance: a new, simplified phenotyping assay. *Ann Clin Biochem*. 2006;43: 354–360.
34. Warner B, Johnston E, Arenas-Hernandez M, et al. A practical guide to thiopurine prescribing and monitoring in IBD. *Frontline Gastroenterol*. 2018;9:10–15.
35. Yarur AJ, Abreu MT, Deshpande AR, et al. Therapeutic drug monitoring in patients with inflammatory bowel disease. *World J Gastroenterol*. 2014;20:3475–3484.
36. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med*. 2010;362:1383–1395.
37. Roblin X, Boschetti G, Williet N, et al. Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. *Aliment Pharmacol Ther*. 2017;46:142–149.
38. Colombel JF, Ferrari N, Debuysere H, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology*. 2000;118:1025–1030.
39. Yang SK, Hong M, Baek J, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. *Nat Genet*. 2014;46:1017–1020.
40. Zhu X, Wang XD, Chao K, et al. NUDT15 polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chinese patients with Crohn's disease. *Aliment Pharmacol Ther*. 2016;44:967–975.
41. Moriyama T, Yang Y-L, Nishii R, et al. Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. *Blood*. 2017;130: 1209–1212.
42. Wong FC, Leung AW, Kwok JS, et al. NUDT15 variant and thiopurine-induced leukopenia in Hong Kong. *Hong Kong Med J*. 2016;22:185–187.
43. Egan LJ, Sandborn WJ. Methotrexate for inflammatory bowel disease: pharmacology and preliminary results. *Mayo Clin Proc*. 1996;71:69–80.
44. Rojas-Feria M, Castro M, Suárez E, et al. Hepatobiliary manifestations in inflammatory bowel disease: the gut, the drugs and the liver. *World J Gastroenterol*. 2013;19: 7327–7340.
45. Herfarth H, Barnes EL, Valentine JF, et al. Methotrexate is not superior to placebo in maintaining steroid-free response or remission in ulcerative colitis. *Gastroenterology*. 2018;155: 1098–1108.
46. Sorrentino D, Nguyen V, Henderson C, et al. Therapeutic drug monitoring and clinical outcomes in immune mediated diseases: the missing link. *Inflamm Bowel Dis*. 2016;22:2527–2537.
47. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther*. 2011;33:987–995.
48. Harper JW, Sinanan MN, Zisman TL. Increased body mass index is associated with earlier time to loss of response to infliximab in patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2013;19:2118–2124.
49. Gonczi L, Kurti Z, Rutka M, et al. Drug persistence and need for dose intensification to adalimumab therapy; the importance of therapeutic drug monitoring in inflammatory bowel diseases. *BMC Gastroenterol*. 2017;17:97.

50. Ferrante M, Vermeire S, Katsanos KH, et al. Predictors of early response to infliximab in patients with ulcerative colitis. *Inflamm Bowel Dis*. 2007;13:123–128.
51. Baumann A. Early development of therapeutic biologics—pharmacokinetics. *Curr Drug Metab*. 2006;7:15–21.
52. Pallagi-Kunstár É, Farkas K, Szepes Z, et al. Utility of serum TNF-alpha, infliximab trough level, and antibody titers in inflammatory bowel disease. *World J Gastroenterol*. 2014;20:5031–5035.
53. Zitomersky NL, Atkinson BJ, Fournier K, et al. Antibodies to infliximab are associated with lower infliximab levels and increased likelihood of surgery in pediatric IBD. *Inflamm Bowel Dis*. 2015;21:307–314.
54. Rivera R, Herranz P, Vanaclocha F. Clinical significance of immunogenicity in biologic therapy. *Actas Dermosifiliogr*. 2014; 105:1–4.
55. Vande Casteele N. Assays for measurement of TNF antagonists in practice. *Frontline Gastroenterol*. 2017;8:236–242.
56. Wang SL, Ohrmund L, Hauenstein S, et al. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods*. 2012;382:177–188.
57. Papamichael K, Cheifetz AS. Use of anti-TNF drug levels to optimise patient management. *Frontline Gastroenterol*. 2016;7: 289–300.
58. Strand V, Balsa A, Al-Saleh J, et al. Immunogenicity of biologics in chronic inflammatory diseases: a systematic review. *BioDrugs*. 2017;31:299–316.
59. Vermeire S, Gils A, Accossato P, et al. Immunogenicity of biologics in inflammatory bowel disease. *Therap Adv Gastroenterol*. 2018;11:1–13.
60. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol*. 2006;4:1248–1254.
61. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut*. 2010;59: 49–54.
62. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med*. 2003;348:601–608.
63. Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology*. 2009;137:1628–1640.
64. Colombel JF, Sandborn WJ, Allez M, et al. Association between plasma concentrations of certolizumab pegol and endoscopic outcomes of patients with Crohn's disease. *Clin Gastroenterol Hepatol*. 2014;12:423–431.
65. Roblin X, Marotte H, Rinaudo M, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2014;12:80–84.
66. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis*. 2013; 19:2568–2576.
67. Colombel JF, Feagan BG, Sandborn WJ, et al. Therapeutic drug monitoring of biologics for inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:349–358.
68. Lie MRKL, Peppelenbosch MP, West RL, et al. Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment. *Aliment Pharmacol Ther*. 2014;40:1202–1208.
69. Ward MG, Thwaites PA, Beswick L, et al. Intra-patient variability in adalimumab drug levels within and between cycles in Crohn's disease. *Aliment Pharmacol Ther*. 2017;45: 1135–1145.
70. Ward MG, Warner B, Unsworth N, et al. Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors. *Aliment Pharmacol Ther*. 2017;46:150–161.
71. Vande Casteele N, Herfarth H, Katz J, et al. American Gastroenterological Association Institute Technical Review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology*. 2017;153: 835–857.
72. Moore C, Corbett G, Moss AC. Systematic review and meta-analysis: serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. *J Crohns Colitis*. 2016;10:619–625.
73. Vande Casteele N, Feagan BG, Gils A, et al. Therapeutic drug monitoring in inflammatory bowel disease: current state and future perspectives. *Curr Gastroenterol Rep*. 2014;16:378.
74. Ordás I, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2012;10: 1079–1087.
75. Vaughn BP, Sandborn WJ, Cheifetz AS. Biologic concentration testing in inflammatory bowel disease. *Inflamm Bowel Dis*. 2015;21:1435–1442.
76. Zittan E, Kabakchiev B, Milgrom R, et al. Higher adalimumab drug levels are associated with mucosal healing in patients with Crohn's disease. *J Crohns Colitis*. 2016;10: 510–515.
77. Ungar B, Levy I, Yavne Y, et al. Optimizing anti-TNF-alpha therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2016;14:550. e2–557.e2.
78. Colombel JF, Sands BE, Rutgeerts P, et al. The safety of vedolizumab for ulcerative colitis and Crohn's disease. *Gut*. 2017;66:839–851.
79. Rosario M, Fox I, Milch C, et al. Pharmacokinetic/pharmacodynamic relationship and immunogenicity of vedolizumab in adults with inflammatory bowel disease: additional results from GEMINI 1 and 2. *Inflamm Bowel Dis*. 2013;19 (suppl 1):S80; P-140.
80. US Food and Drug Administration. ENTYVIO® (vedolizumab) highlights of prescribing information; 2014. Available at: www.accessdata.fda.gov/drugsatfda_docs/label/2014/125476s000lbl.pdf. Accessed November 11, 2019.
81. Ungar B, Kopylov U, Yavzori M, et al. Association of vedolizumab level, anti-drug antibodies, and alpha4beta7 occupancy with response in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2017;16:697.e7–705.e7.
82. Bian S, Dreesen E, Tang HT, et al. Antibodies toward vedolizumab appear from the first infusion onward and disappear over time. *Inflamm Bowel Dis*. 2017;23:2202–2208.
83. Williet N, Boschetti G, Fovet M, et al. Association between low trough levels of vedolizumab during induction therapy for inflammatory bowel diseases and need for additional doses within 6 months. *Clin Gastroenterol Hepatol*. 2017;15: 1750–1757.
84. Yacoub W, Williet N, Pouillon L, et al. Early vedolizumab trough levels predict mucosal healing in inflammatory bowel disease: a multicentre prospective observational study. *Aliment Pharmacol Ther*. 2018;47:906–912.
85. Al-Bawardy B, Ramos GP, Willrich MAV, et al. Vedolizumab drug level correlation with clinical remission, biomarker normalization, and mucosal healing in inflammatory bowel disease. *Inflamm Bowel Dis*. 2019;25:580–586.
86. Sandborn WJ, Baert F, Danese S, et al. Efficacy and safety of vedolizumab subcutaneous formulation in a randomized trial of patients with ulcerative colitis. *Gastroenterology*. 2019;158: 562.e12–572.e12.
87. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med*. 2016;375:1946–1960.
88. Battat R, Kopylov U, Bessissow T, et al. Association between ustekinumab trough concentrations and clinical, biomarker, and endoscopic outcomes in patients with Crohn's disease. *Clin Gastroenterol Hepatol*. 2017;15:1427.e2–1434.e2.

89. Sandborn WJ, Gasink C, Gao LL, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med*. 2012;367:1519–1528.
90. Adedokun OJ, Xu Z, Gasink C, et al. Pharmacokinetics and exposure response relationships of ustekinumab in patients with Crohn's disease. *Gastroenterology*. 2018;154:1660–1671.
91. Vande Casteele N, Buurman DJ, Sturkenboom MG, et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. *Aliment Pharmacol Ther*. 2012;36:765–771.
92. Gardiner SJ, Gearry RB, Burt MJ, et al. Severe hepatotoxicity with high 6-methylmercaptopurine nucleotide concentrations after thiopurine dose escalation due to low 6-thioguanine nucleotides. *Eur J Gastroenterol Hepatol*. 2008;20:1238–1242.
93. Reinshagen M, Schutz E, Armstrong VW, et al. 6-thioguanine nucleotide-adapted azathioprine therapy does not lead to higher remission rates than standard therapy in chronic active crohn disease: results from a randomized, controlled, open trial. *Clin Chem*. 2007;53:1306–1314.
94. Dassopoulos T, Dubinsky MC, Bentsen JL, et al. Randomised clinical trial: individualised vs. weight-based dosing of azathioprine in Crohn's disease. *Aliment Pharmacol Ther*. 2014;39:163–175.
95. Yarur AJ, Kubiliun MJ, Czul F, et al. Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol*. 2015;13:1118.e3–1124.e3.
96. Tighe D, McNamara D. Clinical impact of immunomonitoring in the treatment of inflammatory bowel disease. *World J Gastroenterol*. 2017;23:414–425.
97. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2013;11:444–447.
98. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology*. 2015;148:1320–1329.
99. D'Haens G, Vermeire S, Lambrecht G, et al. Increasing infliximab dose based on symptoms, biomarkers, and serum drug concentrations does not increase clinical, endoscopic, and corticosteroid-free remission in patients with active luminal Crohn's disease. *Gastroenterology*. 2018;154:1343.e1–1351.e1.
100. Ricciuto A, Dhaliwal J, Walters TD, et al. Clinical outcomes with therapeutic drug monitoring in inflammatory bowel disease: a systematic review with meta-analysis. *J Crohns Colitis*. 2018;12:1302–1315.
101. Leach MW, Rottman JB, Hock MB, et al. Immunogenicity/hypersensitivity of biologics. *Toxicol Pathol*. 2014;42:293–300.
102. Scott FL, Clemons B, Brooks J, et al. Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br J Pharmacol*. 2016;173:1778–1792.
103. Tran JQ, Hartung JP, Peach RJ, et al. Results from the first-in-human study with ozanimod, a novel, selective sphingosine-1-phosphate receptor modulator. *J Clin Pharmacol*. 2017;57:988–996.
104. Namour F, Diderichsen PM, Cox E, et al. Pharmacokinetics and pharmacokinetic/pharmacodynamic modeling of filgotinib (GLPG0634), a selective JAK1 inhibitor, in support of phase IIb dose selection. *Clin Pharmacokinet*. 2015;54:859–874.
105. Mohamed MF, Camp HS, Jiang P, et al. Pharmacokinetics, safety and tolerability of ABT-494, a novel selective JAK 1 inhibitor, in healthy volunteers and subjects with rheumatoid arthritis. *Clin Pharmacokinet*. 2016;55:1547–1558.
106. Klünder B, Mohamed MF, Othman AA. Population pharmacokinetics of upadacitinib in healthy subjects and subjects with rheumatoid arthritis: analyses of phase I and II clinical trials. *Clin Pharmacokinet*. 2018;57:977–988.
107. Sandborn WJ, Su C, Sands BE, et al. Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2017;376:1723–1736.
108. Dowty ME, Lin J, Ryder TF, et al. The pharmacokinetics, metabolism, and clearance mechanisms of tofacitinib, a Janus kinase inhibitor, in humans. *Drug Metab Dispos*. 2014;42:759–773.
109. Lamba JK, Lin YS, Thummel K, et al. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics*. 2002;12:121–132.
110. Lawendy N, Lamba M, Chan G, et al. The effect of mild and moderate hepatic impairment on the pharmacokinetics of tofacitinib, an orally active Janus kinase inhibitor. *Clin Pharmacol Drug Dev*. 2014;3:421–427.
111. Mukherjee A, D'Haens GR, Sandborn WJ, et al. Pharmacokinetics and exposure-response of tofacitinib in a phase 3 maintenance study in ulcerative colitis patients. *J Crohns Colitis*. 2017;11(suppl 1):S69–S70; DOP074.
112. Ordás I, Mould DR, Feagan BG, et al. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther*. 2012;91:635–646.
113. Hutmacher MM, Krishnaswami S, Kowalski KG. Exposure-response modeling using latent variables for the efficacy of a JAK3 inhibitor administered to rheumatoid arthritis patients. *J Pharmacokinet Pharmacodyn*. 2008;35:139–157.