The Next Generation of Monitoring Adalimumab Drug and Antibody Levels
Inflammatory bowel diseases (IBDs) are chronically relapsing intestinal inflammatory conditions, of which the most common are Crohn’s disease (CD) and ulcerative colitis (UC). Their etiopathogenesis has not been clearly elucidated, but accumulating evidence suggests that IBD represents an aberrant mucosal immune response to commensal gut organisms in a genetically susceptible host. Genes that contribute to IBD susceptibility appear to control several pathways crucial for intestinal mucosal homeostasis, including the function of the epithelial barrier and regulation of innate and adaptive immunity. The initial breakdown of the intestinal epithelial barrier results in increased bacterial translocation through the lamina propria, where microbial antigens encounter an underlying network of antigen-presenting cells. These cells, which are part of the innate immune system, recognize repetitive motifs on microbial antigens and present antigen fragments to T lymphocytes, leading to secretion of multiple soluble mediators, a majority of which are cytokines and include tumor necrosis factor alpha (TNF), interferon gamma, interleukin-13, and interleukin-17. Subsequent activation of other cell types (e.g., endothelial cells) results in an escalating cycle of cellular trafficking, mediator production, uncontrolled intestinal inflammation, and tissue injury.

TNF is a proinflammatory cytokine that is abundantly expressed in the gastrointestinal tract of patients with IBD. Secreted primarily by activated monocytes and macrophages, TNF amplifies mucosal inflammation through several mechanisms, including disruption of the epithelial barrier, induction of apoptosis in villous epithelial cells, and secretion of chemokines from intestinal epithelial cells. Its proinflammatory activity also extends to upregulating the expression of adhesion molecules and inflammatory mediators on other cell types involved in inflammation, including endothelial cells, neutrophils, macrophages, and immune T and B cells. Because of its central role in IBD pathogenesis, TNF is a logical target for therapeutic intervention and the development of TNF-directed therapies has been a major advance in the treatment of IBD.

Monoclonal antibodies targeting TNF have proven highly effective in managing CD and UC. The anti-TNF agents—infliximab (IFX; Remicade®), Janssen Biotech, Horsham, PA), a chimeric monoclonal antibody against TNF, adalimumab (ADA; Humira®), Abbott Laboratories, Abbott Park, IL), a fully human anti-TNF monoclonal antibody, and certolizumab pegol (Cimzia®), UCB, Inc., Smyrna, GA), a humanized anti-TNF Fab’ monoclonal antibody fragment linked to polyethylene glycol—have demonstrated efficacy for induction and maintenance of remission in patients with moderate-to-severe CD and UC.

Despite the substantial improvement in outcomes that anti-TNF agents have been able to provide for patients with IBD, response is not universal. More than one third of patients do not respond to induction therapy (primary nonresponse) and, even among initial responders, treatment failure occurs in 20% to 60% of patients (secondary nonresponse). These therapeutic failures have not been adequately addressed and pose a challenge to clinicians. Hypotheses for treatment failure that are being actively investigated include the potential role of inadequate serum levels of the anti-TNF agent, consumption of the drug owing to high inflammatory disease burden, and development of immunogenicity (i.e., formation of antibodies to the anti-TNF therapy) during the treatment course. Because of marked interindividual differences in drug pharmacokinetics, bioavailability, and immunogenicity, there is increasing recognition that optimizing treatment according to individual patient needs may provide a more rational therapeutic approach than universal use of a standard regimen derived from large clinical trials in nonuniform patient cohorts. Emerging evidence indicates that higher serum drug levels are associated with longer durations of response and that development of antibodies-to-IFX or -ADA (ATIs or ATAs, respectively) is associated with a diminished or shorter response, suggesting that monitoring anti-TNF serum levels and anti-drug antibody status to inform treatment decisions may result in better outcomes for patients undergoing anti-TNF therapy. A recent review of clinical scenarios using anti-TNF serum levels and anti-drug antibody status to make treatment decisions demonstrated that anti-TNF monitoring in clinical practice has potential in optimizing individual treatment algorithms. However, the clinical utility of such testing has been limited by the sensitivity of currently available test methodologies, including the interference of anti-TNF drug levels with measurement of anti-drug antibodies.

This monograph focuses on adalimumab, one of three anti-TNF agents available for treatment of CD and UC, by presenting the following:

- A brief overview of the efficacy, safety, and tolerability of ADA in patients with IBD.
- Available evidence summarizing the interrelationship between serum ADA levels, ATAs, and disease activity in patients who lose response during ADA therapy.
- A description of a newly available assay—PROMETHEUS® Anser® ADA (Prometheus Laboratories, San Diego, CA)—for the measurement of serum ADA and ATA concentrations, including validation of the assay, its technical advantages over enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA), and its clinical utility for informing the overall treatment of patients with IBD.
Adalimumab

ADA is a subcutaneously administered, recombinant human immunoglobulin G1 (IgG1) monoclonal antibody specific for human TNF, with human-derived heavy and light chain variable regions and human IgG1 kappa constant region (Figure 1).23,24 It is considered “fully human” because the coding gene sequences do not contain elements cloned from other species.25 Even though ADA is a fully human antibody and is less immunogenic than IFX (a human-murine chimera containing 25% murine sequences), the unique antigen-binding site on the variable region can render it immunogenic and capable of provoking an immune response (Figure 2).24,25 The resulting formation of ATAs may reduce the clinical effectiveness of the drug. ADA is approved for the treatment of IBD and several rheumatologic conditions. In CD, ADA is indicated for reducing signs and symptoms and inducing and maintaining clinical remission in adults with moderate to severe disease who have responded inadequately to conventional therapy, have lost response to IFX, or have intolerance to IFX.23 In UC, ADA is indicated for inducing and sustaining clinical remission in adult patients with moderate to severe UC who have had an inadequate response to immunosuppressants such as corticosteroids, azathioprine, or 6-mercaptopurine, but the effectiveness of ADA in patients with loss of response or intolerance to TNF blockers has not been established.23

Overview of Clinical Studies of ADA

A number of pivotal trials establishing the safety and efficacy of ADA for treating patients with IBD resulted in approval of this agent for CD and UC. These studies are reviewed in brief, and the main results are presented here.

Figure 1. Structure of infliximab (IFX) and adalimumab (ADA). IFX and ADA are monoclonal antibodies that bind tumor necrosis factor alpha (TNF-α). IFX is a human-murine chimera that joins the variable regions of a mouse antibody to the constant region of human immunoglobulin G1 (IgG1), and ADA is a human IgG1 antibody. [Reprinted with permission from Anderson.24]
clinical remission were higher after maintenance therapy with ADA dosing every other week and once weekly than with placebo at both week 26 (40% and 47% vs 17%, respectively; \( P < .001 \)) and week 56 (36% and 41% vs 12%, respectively; \( P < .001 \)). Maintenance therapy with weekly dosing of ADA 40 mg offered no advantage over dosing every 2 weeks.\(^{11} \)

In UC, two randomized, double-blind, placebo-controlled studies, ULTRA 1 and 2 (Ulcerative Colitis Long-Term Remission and Maintenance with Adalimumab 1 and 2),\(^{27,28} \) showed that ADA could induce and maintain clinical remission in patients with moderate to severe UC (Mayo score 6–12 on a 12-point scale and an endoscopy subscore of \( \geq 2 \) on a 0 to 3 scale), including those naïve to TNF-blocker therapy and those secondarily unresponsive or intolerant to another TNF blocker. ULTRA 1\(^{27} \) assessed the induction of clinical remission (defined as Mayo score \( \leq 2 \) with no individual subscore \( > 1 \)) at week 8 in 390 patients naïve to TNF-blocker therapy who were randomized to receive ADA or placebo at weeks 0, 2, 4, and 6. ADA was administered in one of two regimens: 160 mg at week 0, 80 mg at week 2, and 40 mg at weeks 4 and 6 or 80 mg at week 0 and 40 mg at weeks 2, 4, and 6. Clinical remission was achieved in 18.5% of patients in the ADA 160/80 mg group, 10.0% in the ADA 80/40 mg group, and 9.2% in the placebo group. The remission rate achieved by the ADA 160/80 group was significant vs placebo (\( P = .031 \)).\(^{27} \)

The second study, ULTRA 2,\(^{28} \) evaluated both induction and maintenance of clinical remission with ADA in 494 patients, 40% of whom had prior exposure to another TNF blocker. Patients were stratified based on prior anti-TNF exposure and randomized to receive ADA (160 mg at week 0, 80 mg at week 2, and 40 mg every other week through week 52) or placebo. Primary end points were the proportions of patients achieving clinical remission at weeks 8 and 52. Remission was achieved in 16.5% of ADA-treated patients by week 8 vs 9.3% of placebo-treated patients (\( P = .019 \)). At week 52, remission rates were 17.3% vs 8.5% (\( P = .004 \)). Remission rates in patients who were anti-TNF naïve significantly favored ADA vs placebo at both week 8 (21.3% vs 11%, respectively; \( P = .017 \)) and week 52 (22% vs 12.4%, respectively; \( P = .029 \)). In patients with a history of prior anti-TNF exposure, no difference in remission rates between ADA and placebo were noted at week 8 (9.2% vs 6.9%, respectively; \( P = .559 \)) but were significant at week 52 (10.2% vs 3%, respectively; \( P = .039 \)).\(^{28} \)

Safety concerns with anti-TNF agents, including ADA, include an increased risk of serious infections resulting in death or hospitalization (including tuberculosis, bacterial sepsis, and invasive fungal and other opportunistic infections), lymphoma, reactivation of hepatitis B virus, new onset or exacerbation of central nervous system demyelinating disease, cytopenia, worsening of congestive heart failure, and formation of autoantibodies.\(^{23} \) Fatal hepatosplenic T-cell lymphoma has been reported in adolescent and young adults with IBD treated concomitantly with anti-TNF agents and immunosuppressants. The main adverse events reported more frequently with ADA than with placebo in controlled clinical trials across all approved indications were injection site reaction, infection, formation of autoantibodies, and liver enzyme elevations. In general, the safety profile of ADA was similar across approved indications, and no safety concerns unique to the use of ADA in IBD were identified.\(^{23} \)

**Figure 2.** Formation of antibodies with infliximab and adalimumab. (A) Human antimouse antibodies are formed in the presence of chimeric monoclonal antibodies, and (B) human antihuman antibodies are formed in the presence of humanized monoclonal antibodies. The antibodies may be neutralizing or non-neutralizing. [Reprinted with permission from Anderson.\(^{24} \)]
**Table 1. Summary of Published Studies on Antibodies to Adalimumab, Adalimumab Levels, and Clinical Outcomes in Crohn’s Disease and Ulcerative Colitis**

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Indication</th>
<th>Regimen and Follow-up</th>
<th>Patients With ATA</th>
<th>ADA Serum Levels (μg/mL)</th>
<th>Impact of ATA on Efficacy</th>
<th>Impact of ADA Levels on Efficacy</th>
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<tbody>
<tr>
<td><strong>Crohn’s Disease</strong></td>
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<tr>
<td>Hanauer et al (CLASSIC I)¹⁰</td>
<td>299</td>
<td>Moderate to severe CD; naïve to anti-TNF therapy</td>
<td>ADA 40/20 mg, 80/40 mg, or 160/80 mg or placebo at wk 0 and 2; follow-up 4 wk</td>
<td>2 patients developed ATAs—Placebo: 1 ATA+ at wk 0; ADA 160/80: 1 ATA+ at wk 2 and ATA– at wk 4</td>
<td>At wk 4—40/20 mg: 2.79±1.48; 80/40 mg: 5.65±3.06; 160/80 mg: 12.61±5.25</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Sandborn et al (CLASSIC II)¹⁴</td>
<td>269</td>
<td>Moderate to severe CD; clinical remission at wk 0 (wk 4 of CLASSIC I) and wk 4</td>
<td>In remission: ADA 40 mg eow or ADA 40 mg/wk or placebo; follow-up 56 wk</td>
<td>7 of 269 patients (2.6%) developed ATAs</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Sandborn et al (GAIN)²⁶</td>
<td>325</td>
<td>Moderate to severe CD; loss of response or intolerance to IFX</td>
<td>ADA 160/80 mg or placebo at wk 0 and 2; follow-up 4 wk</td>
<td>No patient in ADA group ATA+ at wk 4; measurable ADA serum levels precluded determination of ATA</td>
<td>Wk 4—12.6</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>West et al²⁹</td>
<td>30</td>
<td>Active luminal or fistulizing CD; loss of response or intolerance to IFX</td>
<td>ADA 160/80 at wk 0 and 2 and then 40 mg eow (retrospective study); median ADA treatment duration 318 d</td>
<td>5 (17%)</td>
<td>NR</td>
<td>4 of 5 ATA+ patients (80%) were ADA nonresponders; ATA+ was related to ADA nonresponse (OR 13.1; 95% CI 1.7–99.2; P=.006)</td>
<td>NR</td>
</tr>
<tr>
<td>Karmiris et al²¹</td>
<td>168</td>
<td>Luminal or fistulizing CD or arthritic manifestations; loss of response or intolerance to IFX</td>
<td>ADA 160/80 mg or 80/40 mg at wk 0 and 2 or ADA 40 mg eow; median follow-up 20 mo</td>
<td>9.2%</td>
<td>Higher levels with induction dose of 160/80 mg than 80/40 mg</td>
<td>No relationship with short-term response; 11/11 (100%) ATA+ patients had low trough levels of ADA and discontinued ADA</td>
<td>Median trough ADA concentrations lower in ATA+ patients (P&lt;.001)</td>
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<tr>
<td>Bodini et al³⁰</td>
<td>22</td>
<td>CD; IFX naïve; remission with ADA</td>
<td>Maintenance ADA therapy (dosage NR); follow-up 2 yr</td>
<td>9%</td>
<td>Remission; 8.1 (6.7–9.2); disease activity, mild 5.8 (5.2–6.1), moderate 3.9 (3.2–4.7), severe 1 (0.1–2.6) (P&lt;.01 for all)</td>
<td>NR</td>
<td>No effect</td>
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<tr>
<td><strong>Ulcerative Colitis</strong></td>
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<td>Atif et al²¹</td>
<td>20</td>
<td>Moderate to severe UC; IFX naïve or loss of response or intolerance to IFX</td>
<td>ADA 160/80 mg at wk 0 and 2 and then 40 mg eow; follow-up 24 wk</td>
<td>NR</td>
<td>NR</td>
<td>Clinical response to ADA in 5 ATA+ patients (63%) vs 1 ATA– patient (20%)</td>
<td>NR</td>
</tr>
<tr>
<td>Ferrante et al³²</td>
<td>50</td>
<td>Moderate to severe UC; loss of response, infusion reactions, or intolerance to IFX</td>
<td>ADA 160/80 mg at wk 0 and 2 and then 40 mg eow; median follow-up 23 mo</td>
<td>NR</td>
<td>Successful dose escalation associated with median ADA level increase from 4.75 to 7.95 (P=.023)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Sandborn et al (ULTRA 2)²⁸</td>
<td>494</td>
<td>Moderate to severe UC</td>
<td>160/80 mg at wk 0 and 2 and then 40 mg eow or matching placebo; follow-up 52 wk</td>
<td>2.9%, all receiving ADA monotherapy</td>
<td>Median trough ADA levels in remitters vs nonremitters: wk 8 11.4±5.2 vs 8.5±4.4; wk 32 10.6±5.6 vs 7.0±4.0; wk 52: 10.8±7.5 vs 6.2±4.2</td>
<td>NR</td>
<td>NR</td>
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ADA, adalimumab; ATA, antibodies-to-adalimumab; ATI, antibodies-to-infliximab; CD, Crohn’s disease; CLASSIC I and II, Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in Crohn’s disease I and II; eow, every other week; IFX, infliximab; GAIN, Gauging Adalimumab Efficacy in Infliximab Nonresponders study; NR, not reported; UC, ulcerative colitis; ULTRA 2, Ulcerative Colitis Long-term Remission and Maintenance with Adalimumab 2 study.
Clinical Significance of ADA Levels and ATA Formation in IBD

Unlike IFX, ADA has been investigated in only a limited number of studies that evaluated clinical outcomes in IBD in relation to ADA trough levels and the formation of ATAs, owing to the lack of commercially available assays to measure these parameters. These studies are detailed below, and select studies are summarized in Table 1.

In the CLASSIC I trial, 299 patients with moderate to severe CD who were naïve to prior anti-TNF treatment were randomized to receive placebo or one of three induction regimens of ADA subcutaneously at weeks 0 and 2 (160/80 mg, 80/40 mg, or 40/20 mg). Rates of remission (defined as CDAI score <150) at week 4 were significantly higher in the two highest-dose ADA groups (36% and 24%, respectively) than in the placebo group (12%; \( P = .004 \)). Injection site reactions occurred in 38%, 24%, and 26% of patients in the ADA 160/80 mg, 80/40 mg, and 40/20 mg groups, respectively, compared with 16% in the placebo group. Mean ADA serum concentrations reflected the dosing regimen and were higher among patients receiving the highest doses of ADA (12.61 ± 5.25, 5.65 ± 3.06, and 2.79 ± 1.48 μg/mL, respectively). Results from CLASSIC I demonstrate that serum ADA concentrations achieved with the 160/80 mg dose were effective for inducing remission. Only 1 patient in the ADA group developed ATAs, but the authors acknowledged that the short 4-week study may have underestimated the likelihood of developing antibodies. Additionally, the technical limitations of ELISA/ECLIA related to the measurement of ATAs in the presence of ADA increases the rate of inconclusive results, rendering them less useful in terms of informing patient management.

In the CLASSIC II study, which evaluated the long-term efficacy of ADA treatment, 276 participants from CLASSIC I received open-label ADA 40 mg at weeks 0 (week 4 of CLASSIC I) and 2. Patients (\( n = 55 \)) who achieved remission at weeks 0 and 4 were re-randomized to receive ADA 40 mg weekly, ADA 40 mg every other week, or placebo up to week 56. Re-randomized patients with nonresponse or with disease flare could switch to open-label treatment with ADA 40 mg every other week or weekly, if indicated. Patients not in remission at weeks 0 and 4 also received open-label treatment with ADA 40 mg every other week or weekly, if indicated. Among 55 patients randomized at week 4, remission rates at week 56 were significantly higher for the groups receiving ADA 40 mg every other week (15/19, 79%) and ADA 40 mg weekly (15/18, 83%), compared with the group receiving placebo (8/18, 44%; \( P < .05 \) for each ADA group vs placebo). In the open-label cohort of 204 patients not in remission, 93 (46%) were in remission at week 56. Blood samples from 7 of 269 patients tested (2.6%) were positive for ATAs. Of the ATA-positive patients, 3 were in remission at week 24 and 2 were in remission at week 56.

Data from CLASSIC I and CLASSIC II were analyzed by Li et al to evaluate correlations between serum ADA concentrations and clinical remission and to determine if there is a threshold concentration that can reliably predict remission. Serum trough levels of ADA, available for 258 patients, showed a considerable overlap between patients in remission and those not achieving remission. Logistic regression analysis showed a small but statistically significant correlation between ADA levels at week 4 (CLASSIC I) and remission (\( P = .01 \)). In CLASSIC II, serum ADA concentrations did not correlate with remission status at weeks 4, 24, or 56 (\( P = .102, .123, \) and \( P = .091 \), respectively). Threshold analysis did not reveal a serum ADA concentration that was predictive of remission. Thus a dose-exposure relationship was identified for ADA induction, but overlap between groups during maintenance therapy suggests that ADA levels may be of limited value in making therapeutic decisions.

The GAIN study evaluated the efficacy of ADA induction (160 mg at week 0 and 80 mg at week 2) or placebo in 325 patients with moderate to severe CD who had either lost response or were intolerant of IFX. At week 4, remission was achieved in 21% of patients receiving ADA compared with 7% of those receiving placebo (\( P < .001 \)). The mean ADA concentration in ADA-treated patients at week 4 was 12.6 ± 6.0 μg/mL, which was almost identical to the level achieved with ADA 160/80 mg in TNF-naïve patients in CLASSIC I. None of the 159 patients treated with ADA tested positive for ATAs, but this determination may have been compromised by the presence of measurable ADA in serum, which interferes with ELISA/ECLIA determination of ATAs because of their technical limitations.

A limited number of studies have assessed outcomes after ADA treatment in relation to ADA levels and formation of ATAs. A small retrospective study by West et al was the first to demonstrate that the immunogenicity of ADA negatively influenced the response to ADA treatment. Thirty patients with CD previously treated with IFX were subsequently treated with ADA 160 mg at week 0, 80 mg at week 2, and 40 mg every 2 weeks thereafter. The overall clinical response was 77% (23/30 patients). ATAs were detected in 5 patients (17%), of whom 4 were nonresponders. The presence of ATAs was related to nonresponse to ADA (odds ratio [OR] 13.1; 95% confidence interval [CI] 1.7–99.2; \( P = .006 \)). There was no relationship between the presence of ATAs and clinical response in patients with fistulizing disease, whereas ATAs were detected in 75% (3/4) of nonresponders and in only 5.6% (1/17) of responders, and the presence of ATAs was associated with nonresponse in patients with luminal disease (OR 13.5; 95% CI 1.9–98.5; \( P = .01 \)). The presence of ATAs was not related to receipt of concomitant immunosuppression or to a requirement for dose escalation. ATIs were measurable in 57% of patients; serum levels of ATIs were significantly increased in ADA nonresponders compared with responders (163.0 ± 58.6 vs
520.4 ± 244.0 AE/mL, respectively; \( P = .01 \), but no relationship was observed between the presence of ATAs and ATIs. This study was limited by its retrospective design, small size, and measurement of ATAs at varying time points.29

A prospective observational cohort study by Karmiris et al21 evaluated the effect of trough serum ADA concentrations and the presence of ATA during long-term treatment with ADA in 209 patients with CD who lost responsiveness to IFX. Overall, 70.5% of patients (105/149) responded to ADA by week 4 and 61.5% (96/156) showed a sustained clinical benefit. This study reported a higher rate of ATA development—9.2% of patients—than previous studies. Median trough concentrations of ADA were lower in patients who developed ATAs than in patients who did not at both week 4 (2.1 vs 6.1 μg/mL, respectively; \( P < .02 \)) and consistently at all subsequent time points (Figure 3). Over a median follow-up of 20.4 months, lower trough serum ADA concentrations were associated with higher rates of discontinuation (Figure 4). In the 59 (45.4%) patients who discontinued ADA therapy, median trough serum ADA concentration at the time of discontinuation was 3.2 μg/mL. Trough serum ADA concentrations were significantly higher (>6.2 μg/mL at week 4) throughout the study in those patients who continued on therapy compared with those who discontinued (<3.8 μg/mL at week 4). Thus, low trough ADA concentrations were associated with higher early and late discontinuation rates, and a great majority of patients with undetectable serum ADA concentrations also displayed ATAs.21

Bodini et al30 reported findings of an open-label study that prospectively investigated the relationship of clinical outcomes to ADA concentrations and ATA in IFX-naïve patients (N=22) who had achieved remission and received maintenance treatment with ADA over a 2-year period. Clinical activity was assessed using the Harvey-Bradshaw Index (HBI) score and C-reactive protein (CRP) levels. Ten patients (45%) who had sustained clinical remission (HBI <5) at 2 years had significantly higher ADA trough levels than patients with any level of active disease. Median (range) ADA trough concentrations at 2 years were 8.1 μg/mL (6.7–9.2 μg/mL) in patients in remission, 5.8 μg/mL (5.2–6.1 μg/mL) in patients with mild disease, 3.9 μg/mL (3.2–4.7 μg/mL) in patients with moderate disease, and 1 μg/mL (0.1–2.6 μg/mL) in patients with severe disease (\( P < .01 \) via ANOVA).30 Similar to the findings observed by Karmiris et al,21 the 4 patients who lost response and discontinued therapy in this study had significantly lower ADA levels than patients who continued receiving ADA (2.1 vs 6.7 μg/mL, respectively; \( P < .01 \)). ATAs were detected in 9% of patients. In this small study, lower ADA concentrations were associated with loss of response, relapse, and discontinuation of treatment.30

**Figure 3.** Relationship between antibodies-to-adalimumab (ATA) and adalimumab (ADA) trough serum concentrations at different time points. [Reprinted with permission from Karmiris et al.21]

**Figure 4.** Rates of continuation and discontinuation at month 6 based on trough levels at week 4 (A), week 12 (B), and week 24 (C). Top and bottom of box indicate 75th and 25th percentiles, respectively; the band within the box indicates the 50th percentile. [Reprinted with permission from Karmiris et al.]21
To date, the management of loss of response to anti-TNF agents in IBD has included two strategies: one based on results from the determination of anti-TNF drug levels and the presence of anti-drug antibodies and the other based on empiric dose-escalation. The cost implications of these two strategies in patients with CD and secondary nonresponse were unknown until recently. Using a decision analytic model, Velasquez et al. investigated the outcomes of these strategies by comparing two simulated cohorts of patients with CD and examining outcomes in terms of the yield and cost of quality-adjusted life-years (QALYs) gained over a 1-year period.

Results of the model showed that the testing strategy resulted in a cost reduction of $5396 for each QALY gained compared with the cost of each QALY gained using the empiric strategy. Drug level and anti-drug antibody testing resulted in lower proportions of patients receiving high-dose biologics (41% vs 54%, respectively), fewer months receiving high-dose biologic therapy (39% vs 53%, respectively), and more months receiving no biologic therapy (34% vs 19%, respectively) compared with those receiving empiric dose escalation. Thus, the testing strategy provided cost benefits in the biologic therapy management of CD by avoiding the use of this therapy in patients who were unlikely to benefit from it.

In UC, a 24-week uncontrolled trial assessed the benefit of ADA (160 mg at week 0, 80 mg at week 2, and 40 mg thereafter every 2 weeks) in 20 patients who were IFX-naive or who had lost response or developed intolerance to IFX. At week 8, the rate of clinical response (defined as a decrease in the Mayo score >30% from baseline and a decrease of ≥3 points, with a decrease in the rectal bleeding subscore [RBS] ≥1 point or an RBS of 0 or 1) was 25%, and the rate of clinical remission (defined as a Mayo score <2 and no individual score >1) was 5%. Eight of 13 patients (63%) previously treated with IFX were positive for ATIs. A clinical response to ADA was achieved in 5 (63%) of the ATI-positive patients but in only 1 (20%) ATI-negative patient, suggesting that patients who fail IFX secondary to immunogenicity may be more likely to respond to another TNF inhibitor than those who lose response for another reason.

The effect of serum ADA levels on long-term efficacy of ADA (induction with 160/80 mg at 0 and 2 weeks, respectively, followed by maintenance with 40 mg every other week) in patients with UC was studied in 50 patients previously treated with IFX. A short-term clinical response (week 4) was obtained in 68% of patients. Dose escalation was required in 38 (76%) patients either within 6 weeks of initiation (n=20) or because of loss of response after a median of 16 weeks (n=18). In total, 26 (68%) patients responded to dose escalation. Increases in median ADA serum levels from 4.75 to 7.95 μg/mL were noted in patients for whom dose escalation was successful but were not seen in patients with worsening disease. Short-term response and response to dose escalation were associated with colectomy-free survival. The large proportion of patients responding to dose escalation suggests that higher ADA doses may be required in UC.

Despite being fully humanized, ADA is not associated with a complete lack of immunogenicity. This is not surprising because any exogenous protein has the capacity to induce an immune response. The commonly used ELISA and ECLIA methods for detecting ATAs are limited by the interference caused by the presence of drug in serum. Fortunately, a more accurate and sensitive assay has now been developed that overcomes the limitations of these methodologies. As a result, this new assay has the potential to open new doors in the management of IBD by informing treatment decisions for patients receiving ADA.

**Current Assays for Determining ADA Levels and Formation of ATA**

As noted earlier, the incidence of ATA formation in patients treated with ADA for CD or UC is largely unknown. One of the factors contributing to this uncertainty is lack of standardization and methodologic limitations of assays available up to now. In clinical trials in IBD, rates of ATA development after ADA treatment have been reported as high as 17.2% in a retrospective study and 9.2% when assessed prospectively; however, because of drug interference with the assay, this is probably an underestimation. As an example, assay limitations in the UC trials allowed ATAs to be detected only when serum ADA levels were <2 μg/mL; in these patients (25% of the total UC patient population), the rate of detection of ATAs was 20.7%.

In the past, the most commonly used assay to measure antibodies to anti-TNF agents is a solid-phase (double-antigen or sandwich) ELISA in which antibodies in a serum sample are captured on a solid phase coated with a specific anti-TNF agent and detected by binding of biotinylated or peroxidase-coupled anti-TNF. However, limitations may compromise the accuracy and precision of these assays. Measurable anti-TNF drug levels can cause interference and lead to inconclusive results and a potential underestimation of antibodies, problems encountered with the detection of ATAs.

This is especially problematic if serum is collected shortly after administration of the anti-TNF agent; most studies tried to minimize this problem by evaluating trough samples (measured just before the next infusion of anti-TNF). However, the long half-life of ADA may even compromise this strategy. Another confounder is formation of immune complexes between ADA and ATA and their rapid clearance in vivo, which may underestimate both ADA and ATA concentrations and lead to false-negative results. Matrix effects may lead to epitope masking, which prevents detection of functionally monovalent immunoglobulins, such as IgG4, and leads to false-negative results with bridging ELISA. A particular problem with solid-phase ELISAs is false-positive findings caused by nonspecific binding of other
immunoglobulins, rheumatoid factors, or complement factors to the Fc segment of the anti-TNF antibody. ELISA is similar in performance to ECLIA, although the latter uses an electrochemiluminescent label and may be associated with greater sensitivity. However, detection of low-affinity anti-drug antibodies remains problematic with ECLIA owing to sample dilution and a final wash step; ECLIA is also associated with significantly higher costs than ELISA.

To address the shortcomings of currently available assays, a new homogeneous mobility shift assay was developed to quantify anti-TNF antibodies and anti-TNF levels simultaneously in serum samples from patients with IBD. The development, analytical validation, and clinical use of the PROMETHEUS® Anser™ ADA Assay (Prometheus Laboratories, San Diego, CA) for detection of ADA and ATA levels in serum are described in the following sections.

**PROMETHEUS® ANSER™ ADA ASSAY**

The PROMETHEUS® Anser™ ADA Assay is a new, proprietary, nonradiolabeled, fluid-phase assay for detection of ADA and ATA levels. This assay offers several improvements over ELISA/ECLIA; these are summarized in Table 2. The assay has a higher tolerance for ADA in the serum sample compared with current ELISAs and can measure both ADA and ATAs in the same sample.

**Analytical Validation of PROMETHEUS® Anser™ ADA Assay**

The relative ATA concentration was defined as 100 U/mL, equal to 1:100 dilutions. To validate the standard curve, the performance characteristics of the ATA calibration standards were assessed over 29 runs. The coefficient of variation (CV) was <20% for concentrations >0.031 U/mL, and the dynamic range of the assay was 2 orders of magnitude. The calculated limit of detection (LOD) was 0.026 U/mL from the 29 standard curve runs. The lower and upper limits of quantitation were the lowest and highest amounts of an analyte in a sample that could be quantitatively determined with suitable precision and accuracy. Complete analytical validation was performed with the quality control standards by multiple analysts using different instruments on different days. For the PROMETHEUS® Anser™ ADA Assay, intra-assay precision had a CV of <3% and accuracy of <13% error; interassay precision had a CV of <9% and accuracy of <18% error.

The performance characteristics of the PROMETHEUS® Anser™ ADA Assay standard curve were similarly assessed in 29 experimental runs by multiple analysts using different instruments over different days. The CV for all concentrations except the lowest was <25%, and the dynamic range was 2 orders of magnitude. The intra-assay precision was <20% and the accuracy was <3%, whereas the interassay precision was <12% and the accuracy was <22%.

Cut points for ATA and ADA values with the PROMETHEUS® Anser™ ADA Assay were determined from 100 serum samples collected from ADA-naïve, healthy subjects. The calculated cut point for the ATA assay was 0.549 U/mL, with only one sample containing a higher ATA level (100% clinical sensitivity; 99% clinical specificity). Using the same serum samples, the cut point for the ADA assay was 0.676 μg/mL (100% clinical sensitivity; 97% clinical specificity). Further, if a threshold of 1.7 U/mL for the ATA assay or 1.6 μg/mL for the ADA assay is applied, the clinical specificity is increased to 100% [data on file, Prometheus Laboratories, San Diego, CA].

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**Table 2. Methods for Monitoring Antibodies-to-Adalimumab Levels in Patient Serum Samples**

<table>
<thead>
<tr>
<th></th>
<th>Bridge ELISA</th>
<th>PROMETHEUS® Anser™ ADA Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific background interference</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Possibility of false-positive or false-negative</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>IgG4 antibody detection</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ig isotope identification</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tolerance of drug in the sample</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Use and disposal of radioactive material</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; IgG4, immunoglobulin G4.
Potential Interference from Serum Components or ADA

Nonspecific binding of serum substances, which interferes with the accuracy of solid-phase ELISAs, is likely to be minimized in the fluid-phase PROMETHEUS® Anser™ ADA Assay. The potential interference by common endogenous serum components in both ATA and ADA assays was examined by measuring the recovery of ADA and ATAs from spiked quality control samples. Neither assay showed significant interference from physiologic levels of immunoglobulin, rheumatoid factor, hemolyzed serum, or lipemic serum or from the presence of azathioprine (≤10 μM) or methotrexate (≤2 mM).41

Clinical Validation of PROMETHEUS® Anser™ ADA Assay

The ELISA assay method has been used in a research setting to measure the presence of ATA in the serum from patients treated with ADA.21,22 The clinical performance of the PROMETHEUS® Anser™ ADA Assays for measuring ADA and ATAs was assessed with serum samples from 100 patients (with CD, UC, rheumatoid arthritis, or plaque psoriasis) who had received standard ADA therapy for ≥3 months and had achieved and subsequently lost response.41 ADA levels were below the cut point of 0.68 μg/mL in 26% of samples and above 20 μg/mL in 22% of samples. The mean (± standard deviation) ATA level was 4.64±19.20 U/mL, which was significantly higher than the levels recorded in healthy control serum (0.33±0.07 U/mL; P<.00001). Based on an ATA cut point of 0.55 U/mL, 44% of samples were considered ATA positive. ADA levels were inversely related to the presence of ATAs — 68% of serum samples were ATA positive when their ADA levels were lower than the cut point, and 18% were ATA positive when ADA levels were above 20 μg/mL (Figure 5).41

Initial studies evaluated the clinical usefulness of the PROMETHEUS® Anser™ ADA Assay for examining relationships between ATAs, serum ADA levels, and clinical outcomes in patients with IBD. Wolf et al42 performed a cross-sectional study to evaluate mechanisms for loss of response to ADA in 49 patients with CD. Detectable serum ADA levels (median 7.2 μg/mL; range 0.73 – 31.54 μg/mL) were present in 86% (42/49) of patients, and detectable ATAs were present in 47% (23/49). This ATA-positive group would not likely have been detected using standard ELISA/ECLIA testing, because the presence of ADA in the serum would have interfered with ATA identification. Given that ADA has a mean terminal half-life that ranges from 10 to 20 days, if given every 2 weeks, the drug will almost always be present to some degree in the serum of these patients, making ELISA/ECLIA ATA measurements much less useful. ATAs were present in 6 of 7 (86%) patients with undetectable serum ADA. The ADA levels were inversely associated with the occurrence of ATA — the median ADA concentration in ATA-negative samples was 9.78 μg/mL, compared with 4.9 μg/mL in ATA-positive samples (P=.022). This study revealed a high incidence of ATA positivity in patients with loss of response to ADA, as well as an inverse association of ADA levels with ATA generation.42

The PROMETHEUS® Anser™ ADA Assay was used in another cross-sectional study to determine the relationship between ADA and ATAs and inflammation markers and clinical symptoms in patients with IBD.43 Patients were recruited based on use of ADA and not on disease status. In 54 patients, 22.2% (12/54) had detectable ATAs (defined as ≥1 U/mL) and 90.7% (49/54) had detectable ADA (≥1 μg/mL). An ADA concentration ≥5 μg/mL was associated with reduced CRP level (P=.001). Detectable ATA was positively associated with elevated CRP (P=.002) and this correlation was independent of ADA concentration. Patients with low ADA levels (<5 μg/mL) or with detectable ATA had more active disease (P=.01). This study underscored the high prevalence of ATA in IBD patients treated with ADA, as well as the influence of ATA and ADA levels on the inflammatory response. The correlation of active disease with detectable ATA or low ADA levels suggests the clinical usefulness of these assessments.43

The impact of dosing strategies on levels and immunogenicity of ADA was examined by Ben-Bassat et al44 in 57 patients with IBD who received ADA induction therapy followed by three maintenance regimens: 40 mg every other week, dose escalation to 40 mg every week, or dose escalation to 80 mg every other week. Rates of steroid-free clinical remission (HBI ≤2) and normalization of CRP levels (<5 μg/L) were assessed in relation to detectable ADA levels and ATA formation. Serum samples were obtained prior to or at the midinterval...
of maintenance therapy. After a median follow-up of 20.8 months, rates of steroid-free remission were similar between all three maintenance groups (69.2%, 66.6%, and 75.1%, respectively). Median serum ADA levels were similar between the two escalation groups (20.2 μg/mL with 80 mg every other week and 19.1 μg/mL with 40 mg every week) and somewhat higher than with 40 mg every other week (12.5 μg/mL). Overall, 14% of patients had detectable ATAs, with comparable rates of ATA formation between the three maintenance groups (15.3%, 16.6%, and 12.5%, respectively). Median ADA levels were higher among patients in remission than among those with uncontrolled disease (23.8 and 4.9 μg/mL, respectively; \( P < .001 \)) and also higher for patients with normal CRP levels than among those with CRP levels >5 μg/L (22.9 vs 10.5 μg/mL; \( P < .001 \)). Patients who failed treatment had a higher rate of ATA formation (62.5%) and lower median ADA levels (4.2 μg/mL) independent of the maintenance regimen. This study showed both dose escalation regimens to be similar with respect to ADA levels and immunogenicity. Regardless of dosing strategy, treatment failure was associated with lower ADA levels and a higher rate of ATA formation.44

The PROMETHEUS® Anser™ ADA Assay was also used to evaluate the relationship of serum ADA levels and ATAs with indices of serologic and endoscopic mucosal inflammation in another cross-sectional study in IBD.45 Of 66 patients with CD or UC in the study, 62 (94%) had detectable ADA levels and 18 (27%) had detectable ATA levels. An ADA cut point of 5 μg/mL was the best predictor of elevated CRP levels (receiver operator characteristics curve AUC=0.71). The mean ADA level was significantly higher among patients without ATAs than among those with ATAs (12.5 vs 5.7 μg/mL, respectively; \( P = .001 \)) and was also higher among patients with mucosal healing than among those with macroscopic mucosal inflammation (13.3 vs 8.5 μg/mL, respectively; \( P = .02 \)). ADA levels were also higher in patients using an immunomodulator concomitantly than in those receiving ADA monotherapy (14 vs 9 μg/mL, respectively; \( P = .026 \)). The mean CRP level was higher among patients with ATAs than among those without ATAs (12 vs 2.1 mg/dL, respectively; \( P = .002 \)). Patients with detectable ATAs were more likely to have ADA levels <5 μg/mL (OR 8.6; 95% CI 2.3–31.8; \( P < .001 \)), display macroscopic mucosal inflammation (OR 3.8; 95% CI 1.1–13.2; \( P = .03 \)), require steroids (OR 3.7; 95% CI 1.1–12.9; \( P = .03 \)), and to have used IFX previously (OR 3.9; 95% CI 1.0–15.2; \( P = .04 \)). Thus, both ADA and ATA levels correlated with inflammatory activity.46

### Conclusions

Adalimumab is effective in reducing disease in TNF-naïve and TNF-experienced patients with CD or UC, but maintaining long-term response and remission has proven clinically challenging. Increasingly, attention has focused on the immunogenicity of adalimumab and other anti-TNF agents to explain the loss of clinical efficacy over time.17,18,21,46 Although completely humanized, adalimumab is not devoid of immunogenicity, and anti-idiotypic antibodies directed at the variable binding region do develop in adalimumab-treated patients. The presence of antibodies to adalimumab may lead to ineffective subtherapeutic levels of adalimumab and contribute to loss of response by increasing drug clearance or blocking the drug effect. Information on the frequency of anti-adalimumab antibody formation and its influence on response to adalimumab therapy in patients with IBD is limited, but emerging data increasingly highlight the interdependence of the formation antibodies to adalimumab and adalimumab levels in the therapeutic response.21,47,48 Because of the increasing clinical relevance of anti-adalimumab antibodies and adalimumab levels, monitoring these levels during therapy should therefore be integrated into the management of patients receiving adalimumab.

The fluid-phase PROMETHEUS® Anser™ ADA Assay offers several advantages over the solid-phase bridge ELISA/ECLIA assays used for measuring antibodies and anti-TNF levels in serum.41,49 The PROMETHEUS® Anser™ ADA Assay displays high sensitivity, precision, and accuracy. Importantly, the initial acid dissociation step of the PROMETHEUS® Anser™ ADA Assay increases tolerance for free adalimumab in the sample, allowing detection of low levels of ATAs in the presence of levels of adalimumab as high as 20 μg/mL. By avoiding multiple washing steps, the PROMETHEUS® Anser™ ADA Assay can detect antibodies of low affinity, and lack of stereoscopic hindrance allows all immunoglobulin isotypes and subclasses of IgG to be detected. Nonspecific binding is limited because the antigen-antibody reaction occurs in the liquid phase, thereby reducing false-positive results.41,49 The validation data, as well as preliminary studies in IBD patients losing responsiveness to adalimumab, indicate that the assay is effective in measuring ATA in the presence of serum adalimumab.41,43,44 By providing a reliable measure of circulating adalimumab levels and immunogenicity, the PROMETHEUS® Anser™ ADA Assay may be more useful than prior methodologies in informing treatment decisions for optimal clinical outcomes.

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Presented at: Annual United European Gastroenterology Week; October 22–26, 2011; Stockholm, Sweden.


44. Ben-Bassat O, Hauenstein S, Iacono A, Irwin SP, Singh S, Greenberg GR. Serum adalimumab and immunogenicity in IBD patients after 80mg biweekly maintenance therapy [abstract]. Presented at: Annual Digestive Disease Week; May 18–21, 2013; Orlando, FL, USA.


Prometheus diagnostic services provide important information to aid in the monitoring and management of IBD. How this information is used to guide patient care is the responsibility of the physician.

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