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Title Page

Title: Pharmacokinetics and Exposure Response Relationships of Ustekinumab in Patients with Crohn's Disease

Short Title: Ustekinumab pharmacokinetics/dynamics in CD

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Abbreviations: 6-MP, 6-mercaptopurine; AE, adverse event; ANOVA, analysis of variance; AUC, area under the curve; AZA, azathioprine; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; CI, confidence interval; CRP, C-reactive protein; ECLIA,

electrochemiluminescent immunoassay; ER, exposure-response; I, induction; IBD, inflammatory bowel disease; IL12, interleukin 12; IL23, interleukin 23; IV, intravenous; LTE, long-term extension; M, maintenance; MSD, Meso Scale Discovery; MTX, methotrexate; N, number of patients; NA, not applicable; PK, pharmacokinetic(s); Q, quartile; q8w, every 8 weeks; q12w, every 12 weeks; ROC, receiver operating characteristic; SAE, serious adverse event; SC, subcutaneous; SES-CD, Simple Endoscopic Score for Crohn's Disease; TNF, tumor necrosis factor; Y, yes; Yrs, years.

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Abstract

Background and Aims: Ustekinumab is a monoclonal antibody that binds with high affinity to the p40 subunit of human interleukin 12 (IL12 and IL23) that has been approved for treatment of patients with moderate to severe Crohn's disease (CD). However, there are few data on its pharmacokinetic properties or the relationship between drug exposure levels and patient response. We collected data from 2 phase 3 induction studies and 1 maintenance study to determine ustekinumab's pharmacokinetic features, relationship between exposure and response, and optimal serum concentrations for efficacy.

Methods: We collected data on serum concentrations of ustekinumab and efficacy from induction studies of patients with moderate to severe CD given ustekinumab for 8 weeks following a single intravenous dose (either 130 mg or approximately 6 mg/kg). We collected the same data from a maintenance study of patients with a response to ustekinumab in the induction study who then received subcutaneous injections (90 mg) every 8 or 12 weeks for 44 weeks. At week 44 of the maintenance study (52 weeks after treatment began) patients were evaluated for the primary endpoint of clinical remission (defined as a CD activity index score below 150 points), endoscopic markers of efficacy, and serum level of C-reactive protein. Ustekinumab concentration data were categorized into quartiles and relationships between exposure and response were assessed. Optimal concentration cut-off values were evaluated using receiver operating characteristic curve analysis.

Results: Serum concentrations of ustekinumab over time were proportional to dose and did not differ significantly between the induction studies. In the maintenance study, ustekinumab concentration reached the steady state by the second maintenance dose; the median trough concentration was approximately 3-fold higher in patients given ustekinumab at 8-week intervals compared with 12-week intervals. Ustekinumab serum concentrations associated with rates of clinical remission and endoscopic efficacy endpoints, correlated inversely with level of C-reactive protein, and did not associate with use of immunomodulators. Trough concentrations of ustekinumab of 0.8 (or even up to 1.4 µg/mL) or greater were associated with maintenance of clinical remission in a higher proportion of patients than patients with lower trough concentrations.

Conclusions: In an analysis of data from phase 3 studies of patients with moderate to severe CD, we found serum concentrations of ustekinumab to be proportional to dose and associate with treatment efficacy. Concentrations of ustekinumab did not seem to be affected by co-treatment with immunomodulators. Clinicaltrials.gov no:

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Keywords: UNITI trials, inflammatory bowel disease treatment, IBD, anti-IL12/23

INTRODUCTION

Crohn's disease (CD) is a chronic idiopathic inflammatory bowel disease (IBD) that can affect any portion of the intestinal tract¹⁻³ and is histologically characterized by granulomas, fissuring ulceration, submucosal fibrosis, and transmural gut infiltration of lymphocytes and macrophages.^{4, 5} Biologic agents have transformed the treatment of

CD, with tumor necrosis factor (TNF) antagonists at the forefront,⁶⁻¹¹ most often used in patients who do not respond or are intolerant to treatment with corticosteroids and/or oral immunosuppressants.^{12, 13} However, a large proportion of patients with CD either do not respond to treatment with TNF antagonists or only have a transient response that later requires dose escalation or switching to another therapy.^{6, 10, 14-18} Thus, there is a significant medical need for novel, safe, and effective therapies for moderately to severely active CD, particularly in patients who do not respond, lose response, or are intolerant to treatment with TNF antagonists.

In nonclinical studies, the proinflammatory cytokines interleukin 12 (IL12) and interleukin 23 (IL23) have been implicated in the pathophysiology of CD with multiple lines of evidence suggesting that CD is mediated by T_h1 and/or T_h17 cells.¹⁹⁻²⁶ Ustekinumab, a fully human immunoglobulin G1 kappa monoclonal antibody that binds with high affinity to the p40 subunit of human IL12 and IL23, has recently been approved for the treatment of moderately to severely active CD in adults. Ustekinumab prevents IL12 and IL23 bioactivity by preventing their interaction with their cell surface receptor protein IL12Rβ1. Through this mechanism of action, ustekinumab effectively neutralizes IL12 (T_h1)- and IL23 (T_h17)-mediated cellular responses. Evidence for the efficacy of ustekinumab in CD was first supported by the results of a proof of concept Phase 2a study of ustekinumab in patients who had moderate-to-severe CD²⁷ and then in the CERTIFI Phase 2b study of ustekinumab in patients with CD who did not respond or were intolerant to TNF antagonist therapy.²⁸ These Phase 2 studies formed the basis for the Phase 3 program of ustekinumab treatment for patients with CD, which consisted of 2 induction studies (UNITI-1 and UNITI-2) that led into a single randomized

withdrawal maintenance study (IM-UNITI), results for which have been previously reported.²⁹ The UNITI-1 trial included patients who met the criteria for primary or secondary nonresponse to TNF antagonists or had unacceptable side effects, while the UNITI-2 trial included patients in whom conventional therapy failed or unacceptable side effects occurred.

There is currently a lack of ustekinumab pharmacokinetics (PK) and exposure-response (ER) data in CD from large, randomized, controlled trials. These data are critical to therapeutic drug monitoring, which is an important area of focus for gastroenterologists treating IBD. Here, we report on ustekinumab PK and ER relationships using data derived from these previously reported Phase 3 induction and maintenance studies in CD (3 randomized, placebo-controlled, double-blind trials), which comprised the largest cohort to date of ustekinumab-treated patients with CD.²⁹ Understanding the PK and association of ustekinumab exposure to efficacy outcomes and identification of optimal concentration thresholds may ultimately further the ability to individualize treatment of patients with CD.

MATERIALS AND METHODS

Patients and Study Design

Detailed design and clinical results of the UNITI-1, UNITI-2, and IM-UNITI trials have been reported.²⁹ Briefly, all three trials were Phase 3, randomized, double-blind, placebo-controlled, parallel-group, multicenter studies. UNITI-1 and UNITI-2 were 8-week induction studies in patients with moderately to severely active CD. Clinical responders to ustekinumab at Week 8 of induction treatment from UNITI-1 and UNITI-2

comprised the primary analysis population in IM-UNITI, a 44-week randomized-withdrawal maintenance study. The Institutional Review Board or ethics committee at each study site approved the protocols, and all patients provided written informed consent. All authors had access to the study data and have reviewed and approved the final manuscript.

The UNITI-1 trial studied patients with moderate-severe CD (CD Activity Index [CDAI] score of 220 to 450) who previously did not respond, lost response, or were intolerant to TNF antagonists (N=741). These patients were randomized to receive a single intravenous (IV) induction dose of either placebo, a fixed ustekinumab dose of 130 mg, or a tiered dose of ustekinumab approximating 6 mg/kg (260 mg [weight \leq 55 kg], 390 mg [weight >55 kg and \leq 85 kg], or 520 mg [weight >85 kg]), referred to hereafter as ~6 mg/kg. Of the 741 randomized patients, 740 were included in the PK/ER analyses.

In the UNITI-2 trial, patients with CDAI scores of 220 to 450 who had not responded to or were intolerant to conventional therapy (corticosteroids or immunomodulators) but not TNF antagonists (N=628) were randomized to receive the same dosage regimens as the UNITI-1 trial. Of the 628 randomized patients, 68% were naïve to prior TNF antagonists, and 626 of the 628 were included in the PK/ER analyses.

In UNITI-1 and UNITI-2 all patients were evaluated at Week 6 (induction) for the primary endpoint of clinical response, defined as a reduction from baseline in the CDAI score of \geq 100 points; although, patients with a baseline CDAI score of \geq 220 to \leq 248 were considered to be in clinical response if a CDAI score of <150 was attained. The

first major secondary endpoint of clinical remission at Week 8 was defined as a CDAI score of <150 points.

In the IM-UNITI trial, patients in the primary efficacy population were responders to ustekinumab induction in either UNITI-1 or UNITI-2 (N=397) and were randomized 1:1:1 at Week 0 of that study to receive subcutaneous (SC) placebo or 1 of 2 ustekinumab maintenance regimens (ustekinumab 90 mg SC every 12 weeks [q12w] through Week 36 [ie at Weeks 0, 12, 24 and 36] or ustekinumab 90 mg SC every 8 weeks [q8w] through Week 40 [ie at Weeks 0, 8, 16, 24, 32, and 40]). Of the 397 randomized patients, 387 were included in the PK/ER analysis. At Week 44 of that study, 52 weeks after induction Week 0, patients were evaluated for the primary endpoint of clinical remission, defined as a CDAI score of <150 points. ER was also assessed at Week 24 as it was the only concurrent trough time point for both the q8w and q12w ustekinumab regimens. A patient disposition diagram is shown in Supplemental Figure 1 and a study design schematic is shown in Supplemental Figure 2.

Study Evaluations and Analyses

Serum ustekinumab concentrations (hereafter referred to as ustekinumab concentration[s]) were measured in blood samples collected at scheduled visits through Week 8 of UNITI-1 and UNITI-2 and through Week 44 of IM-UNITI (Supplemental Table 1) using a validated electrochemiluminescent immunoassay (ECLIA) method on the Meso Scale Discovery (MSD[®]) platform (Gaithersburg, MD, USA). The lowest quantifiable concentration in a sample for the ECLIA method using

the MSD platform was 0.1688 $\mu\text{g/mL}$ (data on file). Because steady state was invariably attained by Week 24 of the maintenance study for both ustekinumab maintenance regimens, average trough concentrations through Week 44 were obtained by computing the arithmetic mean of patients observed trough concentrations (q12w: Weeks 24 and 36; q8w: Weeks 24, 32, and 40). The relationship between clinical remission and trough ustekinumab concentration quartiles in maintenance were also examined at Week 24 of IM-UNITI, the timepoint where q8w and q12w shared a preadministration trough.

To assess disease activity, CDAI scores were used to determine clinical response and clinical remission, as defined above. Additionally, endoscopic endpoints were assessed at Week 44 (maintenance) using the Simple Endoscopic Score for Crohn's Disease (SES-CD)³⁰ in the subset of patients participating in the endoscopy substudy. Briefly, the scoring of the video endoscopies for the SES-CD was performed by a single reader at a central facility who was blinded to treatment group. Patients with a baseline SES-CD score ≥ 3 (indicating mucosal ulceration in at least one segment) were included in the endoscopy analyses.

The association between ustekinumab concentration and serum C-reactive protein (CRP) was also evaluated. CRP was measured with a validated high-sensitivity CRP immunonephelometry assay using the Siemens BNII Nephelometer (Covance Central Laboratory Services) with a lower limit of quantification of 0.2 mg/L.

Antibodies to ustekinumab were assessed at baseline and Week 6 of the induction study, and at Weeks 0, 12, 24, 36, and 44 of the maintenance study. These analyses were performed using a validated and drug-tolerant ECLIA on the MSD platform (Gaithersburg, MD), in which ustekinumab was used to capture and detect

induced immune responses to ustekinumab. The assay can detect anti-ustekinumab antibodies in the presence of up to 100 µg/mL of ustekinumab. Patients were classified as positive if antibodies were detected at any time in their serum sample.

Statistical Analysis

All ustekinumab concentration data were summarized for each treatment group using descriptive statistics that were calculated at each sampling timepoint. Missing ustekinumab concentration data were not imputed, and the data handling rules for the efficacy variables were previously described in Feagan et al 2016.²⁹ The relationships between ustekinumab concentration and clinical remission and CRP concentration were assessed. A one-sided Cochran-Armitage trend test was used to evaluate the presence of a trend in the proportion of patients with a clinical efficacy outcome across ustekinumab concentration quartiles. For comparisons of variables across concentration quartiles, a nonparametric one-way analysis of variance (ANOVA) based on the median score was used for continuous and ordinal variables, while a Fisher's exact test was used for categorical variables. Optimal cutpoints of ustekinumab concentration associated with efficacy outcomes were determined using receiver operating characteristic (ROC) curve analysis. All statistical testing was performed at the .05 significance level.

RESULTS

Baseline patient characteristics

Among the 1366 UNITI-1 and UNITI-2 patients included in the PK/ER analyses (Supplemental Figure 1), baseline demographic and other characteristics were

representative of an adult population with moderately to severely active CD and were balanced between the treatment groups (Supplemental Table 2 and Table 1).

Pharmacokinetics

All induction dose groups had peak median ustekinumab concentrations at Week 0 (induction), 1 hour after IV infusion. Ustekinumab concentrations were proportional to dose and similar between UNITI-1 and UNITI-2 (Figure 1).

In the UNITI-1 trial median peak ustekinumab concentrations 1-hour post infusion at Week 0 (induction) were 43.6 $\mu\text{g/mL}$ and 129.1 $\mu\text{g/mL}$ for the 130 mg and ~6 mg/kg dose groups, respectively. At Week 8, median ustekinumab concentrations were 2.1 $\mu\text{g/mL}$ and 6.4 $\mu\text{g/mL}$ for the 130 mg and ~6 mg/kg dose groups, respectively.

In UNITI-2, median peak ustekinumab concentrations 1-hour post infusion at Week 0 (induction) were 39.8 $\mu\text{g/mL}$ and 124.4 $\mu\text{g/mL}$ for the 130 mg and ~6 mg/kg dose groups, respectively. At Week 8, median ustekinumab concentrations were 2.0 $\mu\text{g/mL}$ and 6.3 $\mu\text{g/mL}$ for the 130 mg and ~6 mg/kg dose groups, respectively.

Among IV ustekinumab responders randomized to placebo maintenance, median ustekinumab concentrations were undetectable by Week 12 in recipients of 130 mg and by Week 16 in those that had received the ~6 mg/kg induction dose (Figure 2). In contrast, median ustekinumab concentrations were maintained above detectable limits through Week 44 of IM-UNITI among patients randomized to either ustekinumab maintenance regimen (Figure 2). Ustekinumab concentrations reached steady state by the second SC maintenance dose (ie 16 weeks after induction [Week 8 of IM-UNITI] for q8w and 20 weeks after induction [Week 12 of IM-UNITI] for q12w). Median

pre-administration ustekinumab concentrations were consistent through Week 44 for both 90 mg q8w (ranging from 2.0 µg/mL to 2.2 µg/mL at IM-UNITI Weeks 8, 16, 24, 32, and 40) and 90 mg q12w (ranging from 0.6 µg/mL to 0.8 µg/mL at IM-UNITI Weeks 12, 24, and 36) (Figure 2). Thus, median trough concentrations in the ustekinumab 90 mg q8w group were approximately 3-fold greater than in the 90 mg q12w group.

Serum ustekinumab concentrations were similar between patients who were on azathioprine (AZA), 6-mercaptopurine (6-MP), or methotrexate (MTX), compared with those who were not on these drugs (Supplemental Figure 3).

Exposure-response: ustekinumab concentrations and efficacy outcomes

To assess the relationship between efficacy and systemic exposure to ustekinumab, concentrations of ustekinumab at induction Week 8 and maintenance Weeks 24, as well as average trough concentrations through Week 44 were categorized into quartile groups. The proportions of patients in clinical remission (CDAI score <150 points) were summarized by these quartiles.

In both induction trials, combining the 130 mg and ~6 mg/kg dose groups, higher remission rates were observed in the 2 higher ustekinumab concentration quartiles at Week 8, compared with the 2 lower quartiles (Figure 3A and B) though this pattern was more apparent in UNITI-2. This trend was significant in both UNITI-1 ($P = .039$) and UNITI-2 ($P = .007$). Of note, when examining ER within the approved ~6 mg/kg dose, the remission rate remained largely unchanged in UNITI-2 with only a ~1% increase in remission rate from the 3rd to the 4th quartile despite concentration doubling (Supplemental Figure 4B). Although the ER across the quartiles was not as clear in the

UNITI-1 data, the remission rates were not higher in the 3rd or 4th quartile when compared to the 2nd quartile, which had substantially lower concentrations (Supplemental Figure 4A). Taken together, these data do not suggest a higher induction dose would have resulted in higher remission rates at Week 8 across the population.

In the maintenance study, greater proportions of patients were in clinical remission at Week 24 in the higher ustekinumab concentration quartiles (Figure 3C). Clinical remission was seen in 55.3% and 70.8% of patients in the two lower quartiles, while the two higher quartiles saw remission rates of 77.1% and 81.3% ($P = .002$). In the lowest quartile, where the lowest remission rates were observed, a substantial majority of patients were receiving the q12w regimen.

When maintenance ER was examined by dosing regimen (q12w and q8w), the proportion of patients in remission was incrementally higher from the 2nd through the 4th quartile for the q12w treatment quartiles (Figure 3D, $P = .084$). By contrast, the top three quartiles of the q8w treatment all had comparable remission rates around 80% (Figure 3E, $P = .006$). Average steady-state trough concentrations were similarly associated with remission at Week 44 (maintenance primary endpoint; Supplemental Figure 5, $P = .003$). With respect to endoscopic endpoints at Week 44 of maintenance, greater proportions of patients achieved a reduction in the SES-CD score of 3 points or more with increasing ustekinumab concentration (Figure 4A, $P = .038$). Further, greater endoscopic response (Figure 4B, $P = .006$) and remission rates (Figure 4C, $P = .054$) were observed in the top 3 concentration quartiles compared to the first quartile. In all cases, the lowest serum ustekinumab concentration quartile ($\leq 0.5 \mu\text{g/mL}$) had notably lower proportions achieving the endoscopic endpoints.

To assess the relationship between ustekinumab exposure and CRP concentration, the distribution of the CRP concentration was also compared across the ustekinumab concentration quartiles. A trend toward lower CRP concentration with increasing ustekinumab concentration was observed at induction Week 8 (Supplemental Figure 6A and B, $P < .001$). Similar patterns were observed during maintenance with steady-state ustekinumab concentration and CRP at Week 24 ($P < .001$, Supplemental Figure 6C) and Week 44 ($P = .008$; Supplemental Figure 6D). In addition, a greater proportion of patients had normalized CRP at higher serum ustekinumab concentration ($P < .001$, Supplemental Figure 7). However, as seen in Table 1, baseline CRP concentrations were significantly higher to begin with in the lower concentration quartiles (and were also much lower to begin with in the higher quartiles; Table 1). Additionally, a positive correlation was observed between CRP concentration at baseline and at end of induction (Week 8: correlation coefficient $r = .58$, $P < .001$) as well as during maintenance (Week 24: $r = .63$, $P < .001$; Week 44: $r = .68$, $P < .001$). To correct for this, CRP ER analyses were limited to patients with baseline CRP between 3 mg/L and 10 mg/L, a range chosen to eliminate outliers but still include enough patients to analyze (as most patients are in this range). Interestingly, no statistically significant trend was observed in this subgroup analysis suggesting that patients with higher baseline CRP may be driving the association noted in the complete dataset.

Estimation of optimal ustekinumab concentration targets

To identify a concentration of ustekinumab that distinguishes patients with and without clinical remission, ROC curves were generated for remission endpoints during both the induction and maintenance treatment periods. Using serum ustekinumab concentration at Week 8 to correlate with remission at Week 8, the ROC analysis identified a cut-off of 3.3 $\mu\text{g/mL}$ with an area under the curve (AUC) of 0.57 ($P = .001$); sensitivity and specificity were 0.63 and 0.52, respectively (Figure 5A). With respect to maintenance, ROC analyses performed for the combined q8w and q12w regimens resulted in statistically significant AUCs (95% CI = 0.64 [0.56 - 0.70], $P = .003$ using trough concentration at Week 24 versus remission at Week 24 [Table 2, Figure 5B]; and 95% CI = 0.62 [0.54 - 0.69], $P = .011$ using average trough concentration versus remission at Week 44 [Table 2, Figure 5C]). Similar results were obtained using trough concentration at Week 40 versus remission at Week 44 for the q8w regimen only (Table 2). ROC analysis using only the q12w data (data not shown) resulted in AUCs that were not statistically significant; thus, a cut-off could not be determined based on only this subgroup. Overall, steady-state serum ustekinumab trough concentration targets for clinical remission during maintenance ranged from 0.8 to 1.4 $\mu\text{g/mL}$.

Immunogenicity

A total of 1154 treated patients who received at least 1 dose of ustekinumab during induction or maintenance had appropriate samples for antibody testing. Of those, 27 patients (2.3%) were positive for antibodies to ustekinumab during at least one timepoint through one year. Many of these patients were only positive at a single

timepoint and then had subsequent negative antibody results. Additionally, 20 of the 27 patients had titers at or below 1:800. Seventeen of the 27 (63.0%) were positive for neutralizing antibodies. Of note, in subjects randomized to maintenance, induction responders who continued to receive ustekinumab maintenance therapy had a lower incidence of anti-ustekinumab antibodies (2.7% [7/263]) compared with the 5.3% (7/133) who did not receive continuous ustekinumab maintenance (ie induction responders who went on placebo during maintenance). Among treated patients, the proportion positive for antibodies to ustekinumab was 1.9% (7/375) among those who received concomitant immunomodulators and 2.6% (20/779) among patients who did not. Median serum ustekinumab concentrations were generally lower in the few patients who were positive for antibodies. For example, at Week 24, median steady-state serum ustekinumab concentration was 0.3 µg/mL in the 5 patients positive for antibodies to ustekinumab compared with 1.1 µg/mL in the 186 patients who were negative. None of the patients who were positive for antibodies to ustekinumab had injection-site, serum-sickness-like, or anaphylaxis reactions.

Safety

Select aggregate adverse events (AEs) that occurred during these trials have been previously published.²⁹ No consistent relationship was observed between ustekinumab concentration and the incidence of infections, serious infections, or serious AEs during either induction or maintenance (Supplemental Table 3).

DISCUSSION

Ustekinumab, which was approved for CD in the US, Canada, and EU in 2016, targets the IL12 and IL23 inflammatory pathways and has a well-established safety profile in clinical trials and clinical practice since its first approval for moderate to severe plaque psoriasis in 2009.³¹⁻³³ In the present analyses of the UNITI-1 and UNITI-2 CD induction studies and the IM-UNITI maintenance study, we evaluated the PK of ustekinumab in patients with moderately to severely active CD and provide the first detailed assessments of associations between ustekinumab concentration and efficacy outcomes. This is the most comprehensive PK and ER evaluation of ustekinumab in patients with CD to date. Understanding ustekinumab PK characteristics and the relationship between efficacy outcomes and ustekinumab concentrations is important for prescribers to optimize efficacy with ustekinumab therapy.

These analyses demonstrated that ustekinumab exhibits dose-proportional PK behavior with IV induction doses of 130 mg and ~6 mg/kg that was similar in patients who did not respond to, lost response to, or were intolerant to treatment with TNF antagonists (UNITI-1 population) as well as in patients who did not respond or were intolerant to conventional therapy and who were predominantly naïve to TNF antagonists (UNITI-2 population). These findings suggest that the clearance of ustekinumab does not vary with dose or study patient population. Therefore, the better efficacy seen in TNF-antagonist-naïve patients compared to TNF-antagonist-failure patients does not seem to be attributable to differences in ustekinumab exposure. The observed dose proportional profile of ustekinumab allows for the prediction of the impact of dose changes on systemic exposure.

Strong positive associations were seen between ustekinumab concentration and clinical efficacy outcomes in induction. With the approved ~6 mg/kg dose, remission rates appear to have peaked in the higher concentration quartiles. However, in both induction studies, patients in the lowest quartile demonstrated notably lower rates of remission at Week 8 suggesting these patients with high drug clearance and baseline disease activity are a population that would be appropriate to consider further induction dose intensification, eg, in future studies.

Similarly, maintenance ER analysis showed strong positive correlations between steady-state trough ustekinumab concentrations (first attained at the time of the second 90 mg SC dose) and remission with both maintenance regimens (90 mg SC q12w and q8w) individually, as well as when the data from the regimens were combined. Within the q8w regimen, efficacy appears to have peaked from the 2nd concentration quartile suggesting that an efficacy plateau was reached at the exposures attained by most patients. The patients in the lowest quartile (≤ 0.9 $\mu\text{g/mL}$) with this regimen achieved a substantially lower clinical remission rate. Data within the 90 mg q12w group was consistent with this observation as the highest remission rates occurred within the last quartile (> 1.2 $\mu\text{g/mL}$) in the q12w group and was comparable with remission rates in patients who fell within or above the 2nd quartile (> 0.9 $\mu\text{g/mL}$) in the q8w group. Endoscopic outcomes at Week 44 were also notably greater in the 3 higher quartiles (> 0.5 $\mu\text{g/mL}$) than in the first quartile (≤ 0.5 $\mu\text{g/mL}$).

Based on the ROC analyses, steady-state concentration cut-offs ranging between 0.8 to 1.4 $\mu\text{g/mL}$ were associated with greater clinical remission during maintenance, corroborating the quartile analysis. The range of these target

concentrations illustrate that there is some heterogeneity in applying precise level “targets” to individual patients, even when they are valid across a population. In terms of implications for clinical practice, this appears to support shortening the interval to q8w in q12w patients that are not in remission and have trough levels below this range (in regions where q12w is an approved regimen). Patients on 90 mg q8w in this same circumstance would be a potential population in which to evaluate further dose intensification. Ideally, a future prospective study would examine if better clinical outcomes are achieved by interval shortening (eg to 6 or 4 weeks) in order to attain higher trough concentrations above the 0.8 to 1.4 µg/mL target.

In contrast with the above findings about ustekinumab concentration thresholds for optimal efficacy, Battat et al³⁴ recently reported that trough levels above 4.5 µg/mL were associated with biomarker reduction and endoscopic response in a TNF antagonist-refractory population. Importantly, the study that identified this relatively high trough level was limited by a small sample size (N=62), the use of SC administration for induction, and the fact that the majority of the patients (approximately 75%) received ustekinumab 90 mg every 4 weeks as opposed to the approved q8w or q12w regimens. This is relevant because levels are invariably higher at 4 weeks, specifically, >2-fold higher than at 8 weeks given ustekinumab’s ~3-week half-life. In addition, potential differences in the assays used to measure ustekinumab may have contributed to the apparent discrepancy in the proposed concentration threshold. Of the factors evaluated, body weight, serum albumin, and disease severity indicators (CDAI, fecal markers, CRP) were associated with differences in ustekinumab concentration.

These findings are consistent with reports of the PK characteristics of some other monoclonal antibodies used in the treatment of inflammatory bowel disease.^{35, 36}

The incidence of antibodies to ustekinumab through 1 year on treatment was 2.3% (using a drug-tolerant assay), indicating that ustekinumab has low immunogenicity. In contrast, the rates of anti-drug antibodies with similarly drug-tolerant assays with TNF antagonists are substantially higher with rates of 39.8% for adalimumab³⁷ and 51% for infliximab³⁸ in recent reports using drug-tolerant assays. Although lower serum concentrations were observed among those who had antibodies to ustekinumab, there was no demonstrable effect of immunogenicity on efficacy; however, with such a low proportion of patients exhibiting anti-drug antibodies, such associations cannot be fully assessed. Importantly, the incidence of anti-drug antibodies was slightly higher among those who were randomized to placebo maintenance (5.7%), suggesting that intermittent therapy is a risk factor for immunogenicity, as has been seen with other biologics.³⁹

In contrast to the experience with TNF antagonists,^{7, 40} there was no significant impact of AZA, 6-MP, or MTX on serum ustekinumab concentration and immunogenicity. For TNF antagonists such as infliximab, the effects of these drugs on PK is hypothesized to be the result of decreased immunogenicity (ie, less tendency to develop ADAs), a possible shared mechanism of apoptosis,⁷ or a decreased expression of receptors important for monoclonal antibody disposition (for example, Fcγ receptors on monocytes thereby affecting a monoclonal antibody's PK).⁴¹⁻⁴³ In the case of ustekinumab treatment in patients with CD, the low incidence of anti-ustekinumab antibodies may explain the lack of impact of AZA, 6-MP, or MTX on ustekinumab levels,

supporting immunogenicity as the primary driver of the impact of such drugs on antibody concentration. In contrast to other biologics, because of the apparent lack of need for an immunomodulator, it would seem appropriate to utilize ustekinumab as monotherapy in CD rather than combination therapy.

In addition to the association of systemic ustekinumab exposure and clinical efficacy variables, we found that ustekinumab concentration was inversely related to CRP concentration and positively correlated with normalization of CRP during both induction and maintenance. While this points to the possibility that CRP concentration could provide some indication of the effect of treatment, it is difficult to interpret due to the observation that post-treatment CRP is highly correlated with pre-treatment CRP. This implies that drug clearance (and therefore ustekinumab concentration) is associated with baseline CRP. This is presumably a function of the higher underlying disease activity, for which CRP is a marker, rather than the CRP molecule itself. Thus, pre-treatment CRP may be a potential predictor of ustekinumab concentration in patients receiving ustekinumab.

Regarding safety, we did not observe an association between ustekinumab concentration and infections, serious infections, or serious adverse events. This finding suggests that the occurrence of these safety events is not attributable to the levels of ustekinumab exposure attained with the dose regimens evaluated in these Phase 3 studies.

It is important to acknowledge that these analyses had some limitations. First, the number of patients in the maintenance study decreased over time, which may have implications for the maintenance ER analysis. However, ER analysis using

model-predicted ustekinumab concentration data yielded similar results. Second, the ustekinumab concentration cut-offs obtained from the ROC analyses were based on statistically significant but modest AUC and specificity values which suggest that factors other than serum ustekinumab concentrations (such as markers of inflammatory burden) may need to be evaluated to improve the ability to predict efficacy. In addition, the relatively low specificity associated with the identified thresholds implies that the likelihood of false-positive results (ie, low concentrations occurring in responders) may be high and that additional clinical judgment should be employed if patients appear to maintain efficacy despite low concentrations. On the other hand, the relatively higher sensitivity values associated with these thresholds assure treating physicians that levels above these targets are likely adequate to achieve, or maintain, efficacy. Nevertheless, the predictive ability of drug concentration versus efficacy is consistent with those observed from the ROC analyses of TNF antagonists in IBD.⁴⁴⁻⁴⁶ Finally, while the incidence of antibodies to ustekinumab was low and no impact was observed on efficacy and safety, these results should be interpreted with caution due to the limited number of patients with anti-ustekinumab antibodies in these analyses.

In conclusion, a positive association between ustekinumab concentrations and efficacy outcomes in patients with CD was confirmed during both induction (UNITI-1 and -2) and maintenance (IM-UNITI) studies. The ER findings support the use of both the approved weight-based induction regimen (~6 mg/kg) and the q8w maintenance regimen for the treatment of CD, although a number of patients on the q12w maintenance regimen also attained the ustekinumab concentration cut-off associated with efficacy outcomes. Importantly, ustekinumab concentrations were not influenced by

immunomodulators, in marked contrast to infliximab⁷, suggesting ustekinumab can be used as monotherapy and there may be no benefit or need for combination therapy. While additional studies will be required to determine whether proactive therapeutic drug monitoring to target levels improves long-term CD outcomes, these results from the largest cohort of patients with CD treated with ustekinumab to date (1369 total patients and ~100 on each maintenance regimen) can provide important guidance for treating moderately to severely active CD with ustekinumab.

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Figures

Figure 1. Median serum ustekinumab concentrations illustrating dose proportionality over time during the induction studies UNITI-1 and UNITI-2.

Figure 2. Median serum ustekinumab concentrations over time after induction with a dose of 130 mg (A) or ~6mg/kg (B) and through the maintenance study IM-UNITI. The patients in the placebo groups in both A and B received an IV induction dose of ustekinumab only and were on placebo treatment during maintenance. I, induction;

IV, intravenous; M, maintenance; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous.

Figure 3. Proportions of patients achieving clinical remission by serum ustekinumab concentration quartiles at Week 8 in the UNITI-1 (A) and UNITI-2 (B) induction studies, at Week 24 (C) in the IM-UNITI maintenance study, and at Week 24 in the q12w (D) and q8w (E) doses in the IM-UNITI maintenance study. Patients in the placebo group received an IV induction dose of ustekinumab only and were on placebo treatment during maintenance. IV, intravenous; N, number of patients; Q, quartile; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous.

Figure 4. Proportions of patients achieving endoscopic endpoints by serum ustekinumab concentration quartiles at Week 44 in the IM-UNITI maintenance study. Reduction in the SES-CD score of ≥ 3 points (A), endoscopic response (B), and endoscopic remission (C). Average trough concentrations were obtained by computing the arithmetic mean of the observed trough concentration for each patient (q12w: Week 24, Week 36; q8w: Week 24, Week 32, and Week 40) to reflect average exposure at steady state. N, number of patients; Q, quartile; SES-CD, Simple Endoscopic Score for Crohn's Disease.

Figure 5. Receiver operating characteristic curve analysis of optimal serum ustekinumab thresholds associated with clinical remission at Week 8 (A) of the combined UNITI-1 and UNITI-2 induction studies, and at Week 24 (B) and Week 44 (C) of the IM-UNITI maintenance study. Average trough concentrations were obtained by

computing the arithmetic mean of the observed trough concentration for each patient (q12w: Week 24, Week 36; q8w: Week 24, Week 32, and Week 40) to reflect average exposure at steady state. AUC, area under the curve.

Tables

Table 1: Comparison of Baseline Patient Characteristics by Serum Ustekinumab Concentration Quartiles at Week 8 of Induction and at Week 24 of Maintenance Among Patients Treated with Ustekinumab in the UNITI-1, UNITI-2, and IM-UNITI Studies.

Characteristic						
<u>Baseline values at Induction Week 0</u>						
<u>Median values</u>						
	Ustekinumab concentration at Week 8 (Induction)					<i>P</i> -value ^a
	All	Q1	Q2	Q3	Q4	
N	701	175	175	176	175	
Age (yrs)	36.0	32.0	36.0	37.0	41.0	.004
Body weight (kg)	68.5	66.4	66.8	69.9	72.3	.064
CDAI^b	302.0	324.0	305.0	294.5	292.0	.017
Disease duration from time of diagnosis (yrs)	8.4	6.9	9.1	7.8	10.2	.135
Fecal calprotectin (µg/kg)	473.4	727.8	473.4	374.3	242.3	<.001
Fecal lactoferrin (µg/mL)	88.2	202.4	102.6	61.6	39.3	<.001
Albumin (g/dL)	3.6	3.2	3.6	3.7	3.8	<.001
CRP (mg/L)	8.4	22.5	9.2	5.6	5.3	<.001
<u>Proportions</u>						
Male Gender, %	43.1	48.6	44.6	38.6	40.6	.245
Antibody-to-ustekinumab status (positive), %	3.1	5.7	0.6	2.9	3.4	.042
History of TNF antagonist use, %	64.9	69.1	65.1	65.3	60.0	.359
Concomitant immunomodulator use at baseline (Y), %	33.4	33.1	33.7	34.1	32.6	.993

Corticosteroid use at baseline (Y), %	36.0	38.3	34.9	39.2	31.4	.411
Ustekinumab concentration at Week 24 (Maintenance)						
	All	Q1	Q2	Q3	Q4	P-value ^a
N	191	47	48	48	48	
Age (yrs)	35.0	39.0	31.0	35.0	37.5	.050
Body weight (kg)	67.6	70.5	67.3	64.3	70.9	.318
CDAI ^b	299.0	307.0	310.5	292.5	300.0	.377
Disease duration from time of diagnosis (yrs)	7.0	6.7	8.1	7.0	7.7	.876
Fecal calprotectin	523.3	595.9	550.0	523.5	318.1	.026
Fecal lactoferrin	104.6	172.5	128.4	107.8	36.9	.058
Albumin (g/dL)	3.6	3.4	3.6	3.8	3.9	<.001
CRP (mg/L)	8.0	19.4	10.2	6.9	2.6	<.001
Proportions						
Male Gender, %	44.0	55.3	41.7	33.3	45.8	.187
Antibody-to-ustekinumab status (positive), %	2.6	6.4	2.1	0.0	2.1	.227
History of TNF antagonist use, %	53.9	59.6	45.8	60.4	50.0	.398
Immunomodulator use at baseline (Y), %	39.3	46.8	35.4	41.7	33.3	.532
Corticosteroid use at baseline (Y), %	34.0	31.9	31.3	41.7	31.3	.658

^aFor comparisons of variables across ustekinumab concentration quartiles, a nonparametric one-way ANOVA based on the median score was used for continuous and ordinal variables, while a Fisher's exact test was used for categorical variables.

^bCDAI scores range from approximately 0 to 600, with higher scores indicating worse disease and a 50-point change indicating the minimal clinically important difference.

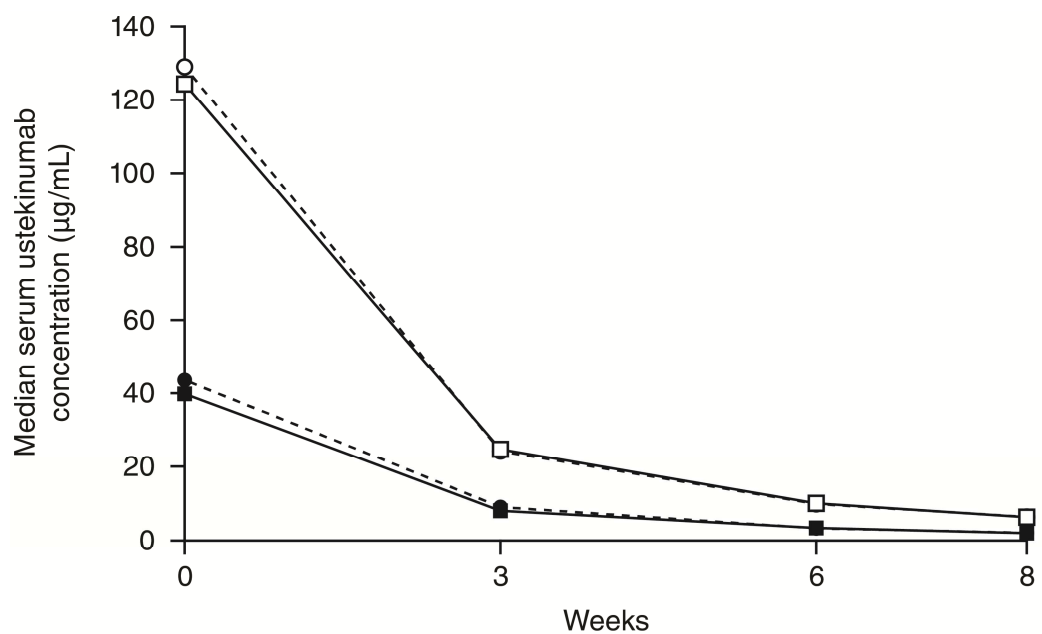
ANOVA, analysis of variance; CDAI, Crohn's Disease Activity Index; CRP, C-reactive protein; N, number of patients; Q, quartile; TNF, tumor necrosis factor; Y, yes; Yrs, years.

Table 2: Receiver Operating Characteristic Analysis Metrics for the Relationship Between Ustekinumab Concentration and Efficacy During the IM-UNITI Study.

PK Measure	Efficacy Endpoint	ROC metric	Value
Trough ustekinumab concentration at Week 24 (Combined q8w and q12w)	Remission at Week 24	AUC	0.64
		(95% CI; <i>P</i> -value)	(0.56 - 0.70; <i>P</i> = .003)
		Sensitivity (%)	67
		Specificity (%)	60
		Threshold (µg/mL)	0.82
Average trough ustekinumab concentration ^a (Combined q8w and q12w)	Remission at Week 44	AUC	0.62
		(95% CI; <i>P</i> -value)	(0.54 - 0.69; <i>P</i> = .011)
		Sensitivity (%)	73
		Specificity (%)	56
		Threshold (µg/mL)	0.80
Trough ustekinumab concentration at Week 40 (q8w only)	Remission at Week 44	AUC	0.66
		(95% CI; <i>P</i> -value)	(0.54 - 0.76; <i>P</i> = .047)
		Sensitivity (%)	82
		Specificity (%)	47
		Threshold (µg/mL)	1.35

^aAverage of trough ustekinumab concentrations at Weeks 24, 32 and 40 for q8w and at Weeks 24 and 36 for q12w.

AUC, area under the curve; CI, confidence interval; PK, pharmacokinetic; q8w, every 8 weeks; q12w, every 12 weeks; ROC, receiver operating characteristic.



UNITI-1

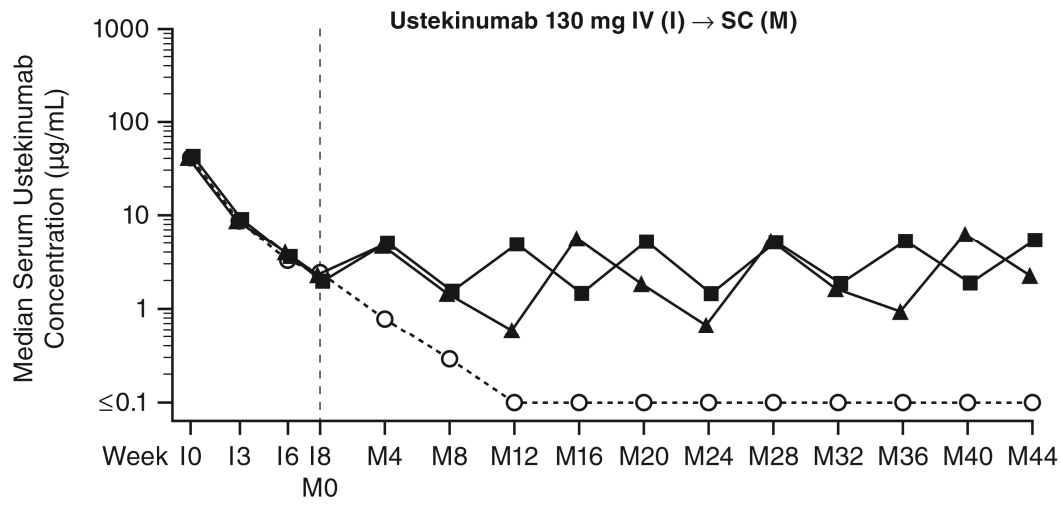
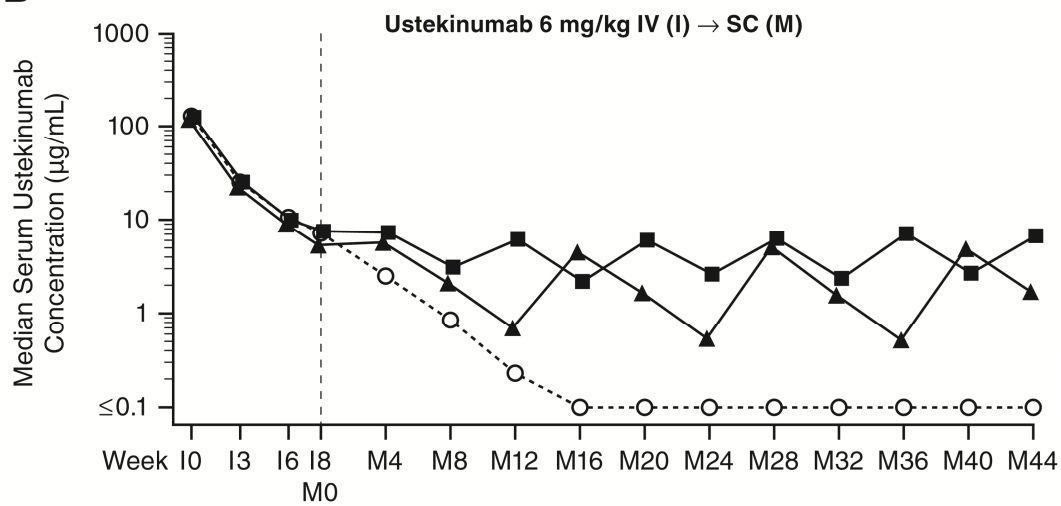
--●-- Ustekinumab 130 mg

--○-- Ustekinumab 6 mg/kg

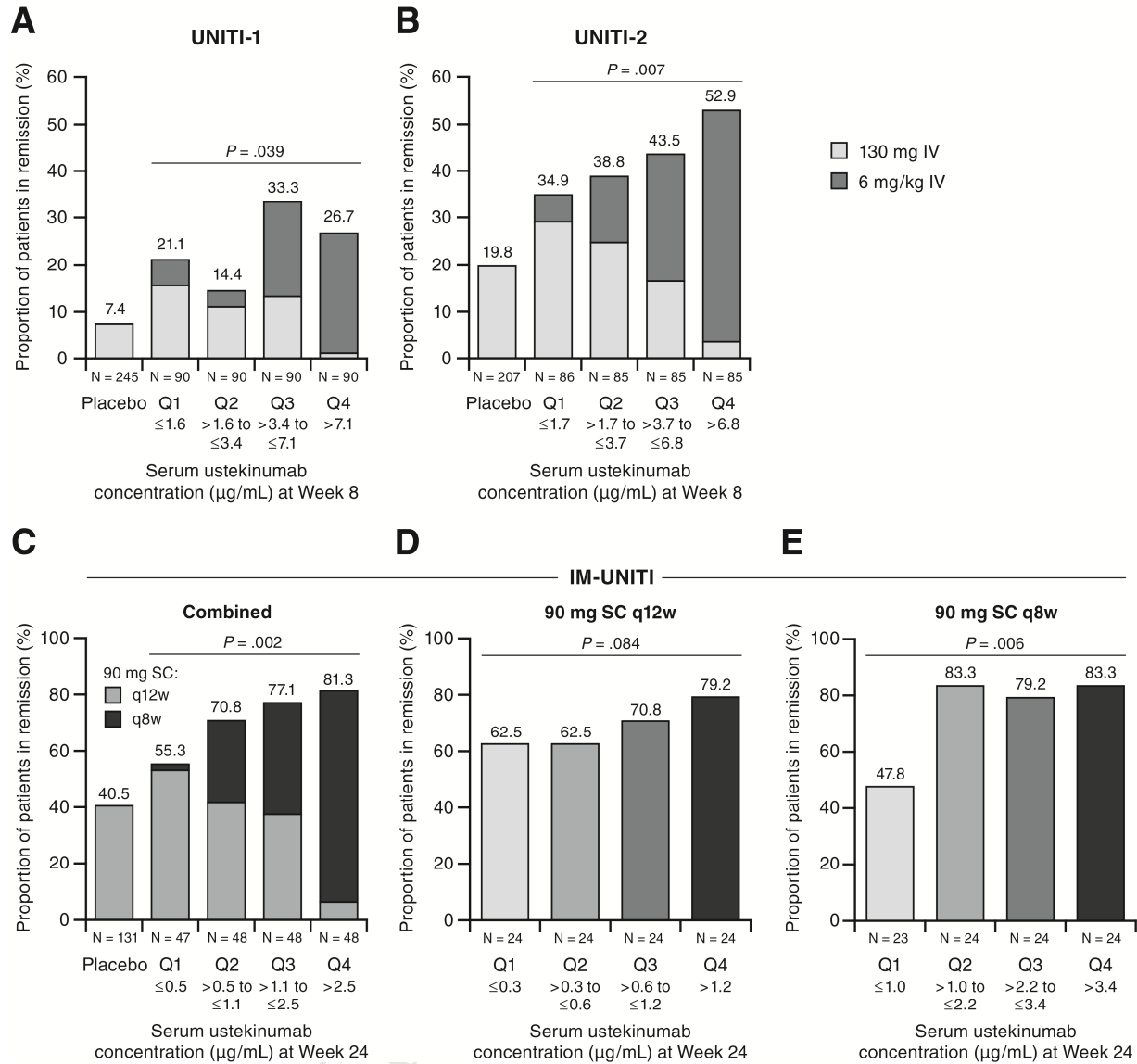
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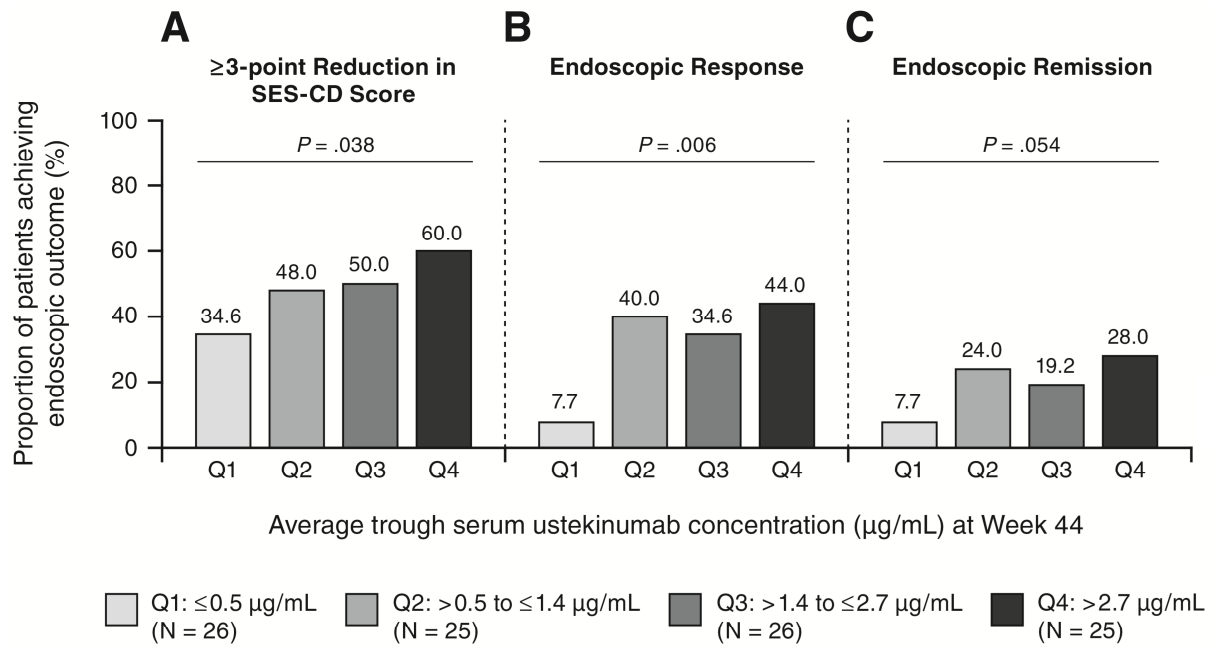
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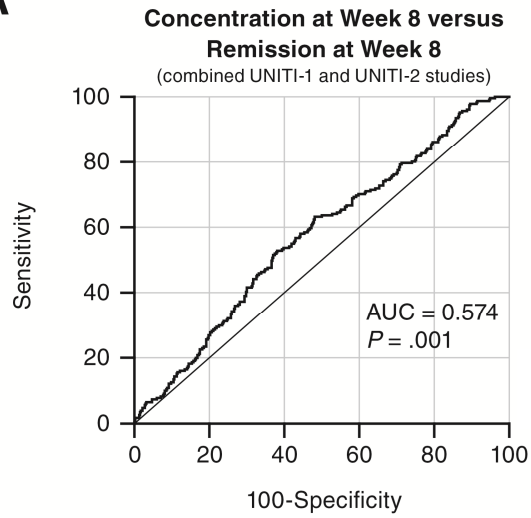
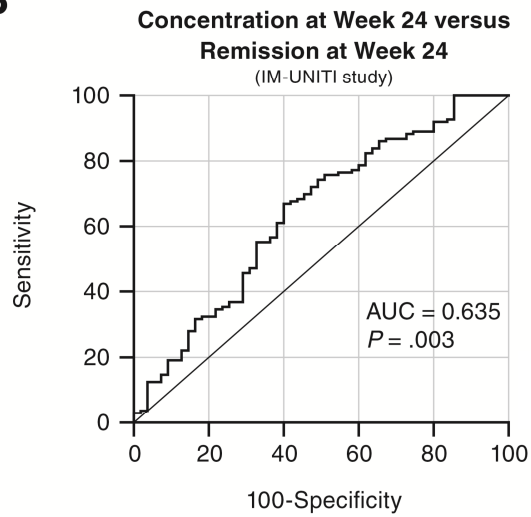
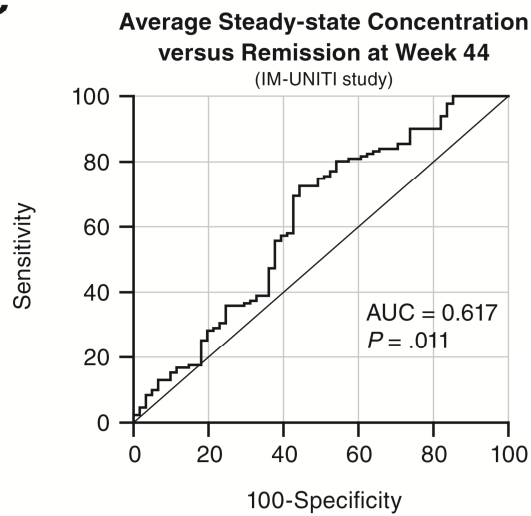
—□— Ustekinumab 6 mg/kg

A**B**

--○-- Placebo ▲ 90 mg SC q12w ■ 90 mg SC q8w





A**B****C**

Supplemental Material

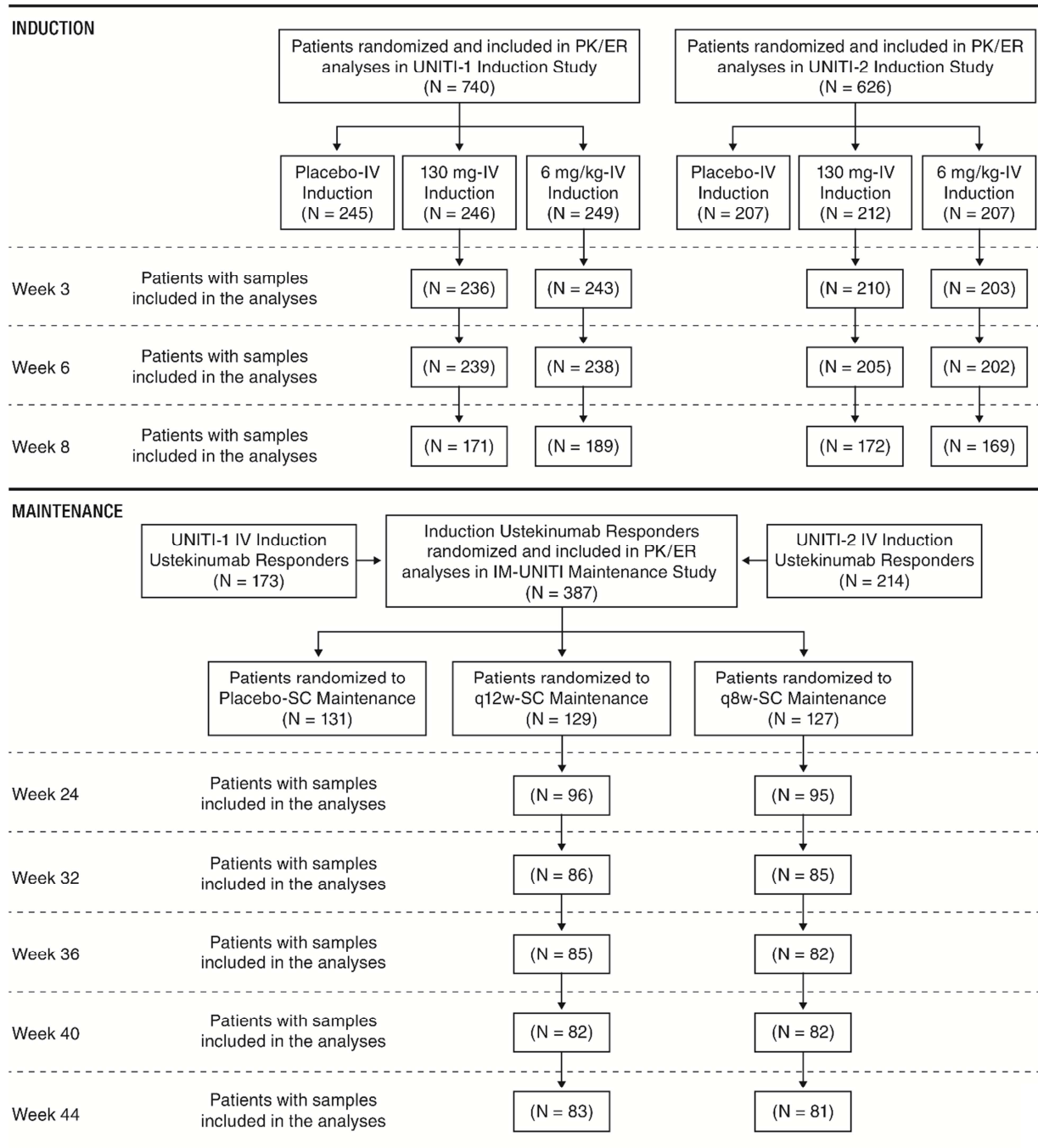
Title: Pharmacokinetics and Exposure Response Relationships of Ustekinumab in Patients with Crohn's Disease

Authors: Omoniyi J Adedokun¹, Zhenhua Xu¹, Christopher Gasink², Douglas Jacobstein¹, Philippe Szapary¹, Jewel Johanns¹, Long-Long Gao², Hugh M Davis¹, Steve Hanauer³, Brian G Feagan⁴, Subrata Ghosh⁵, William J Sandborn⁶

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Supplemental Figures

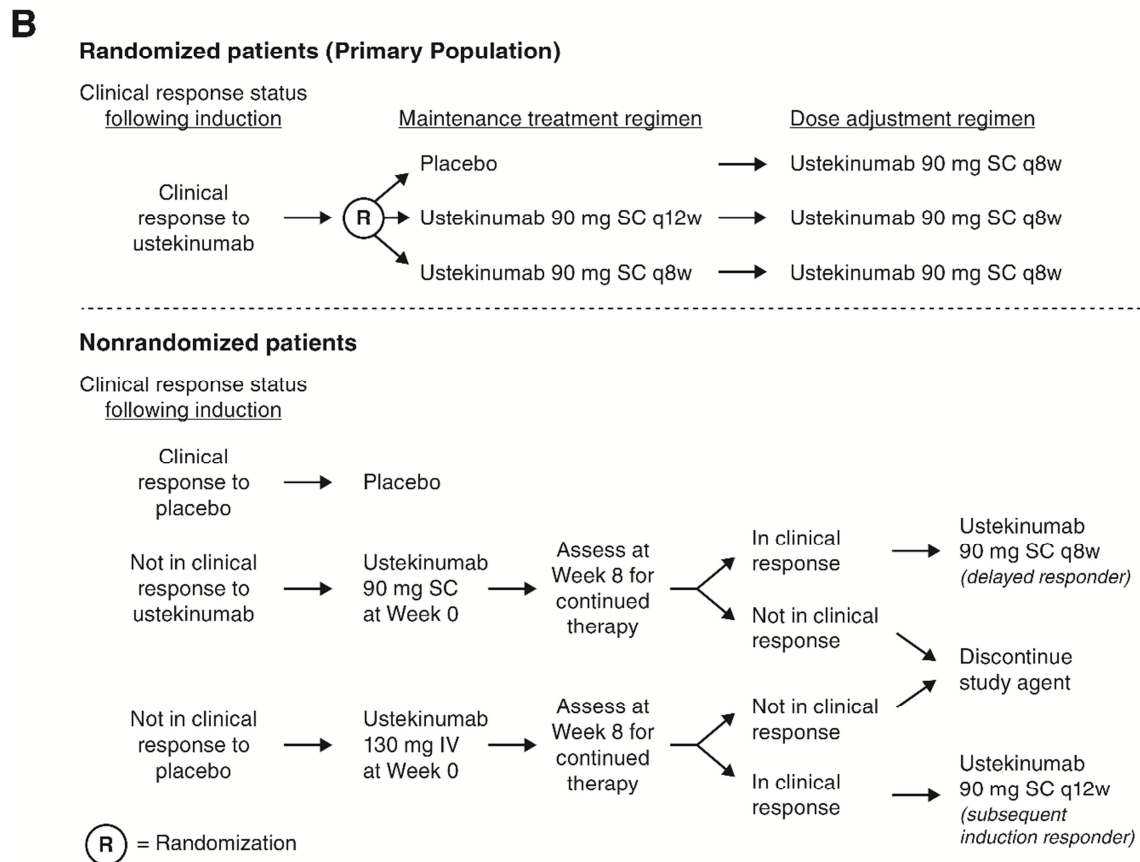
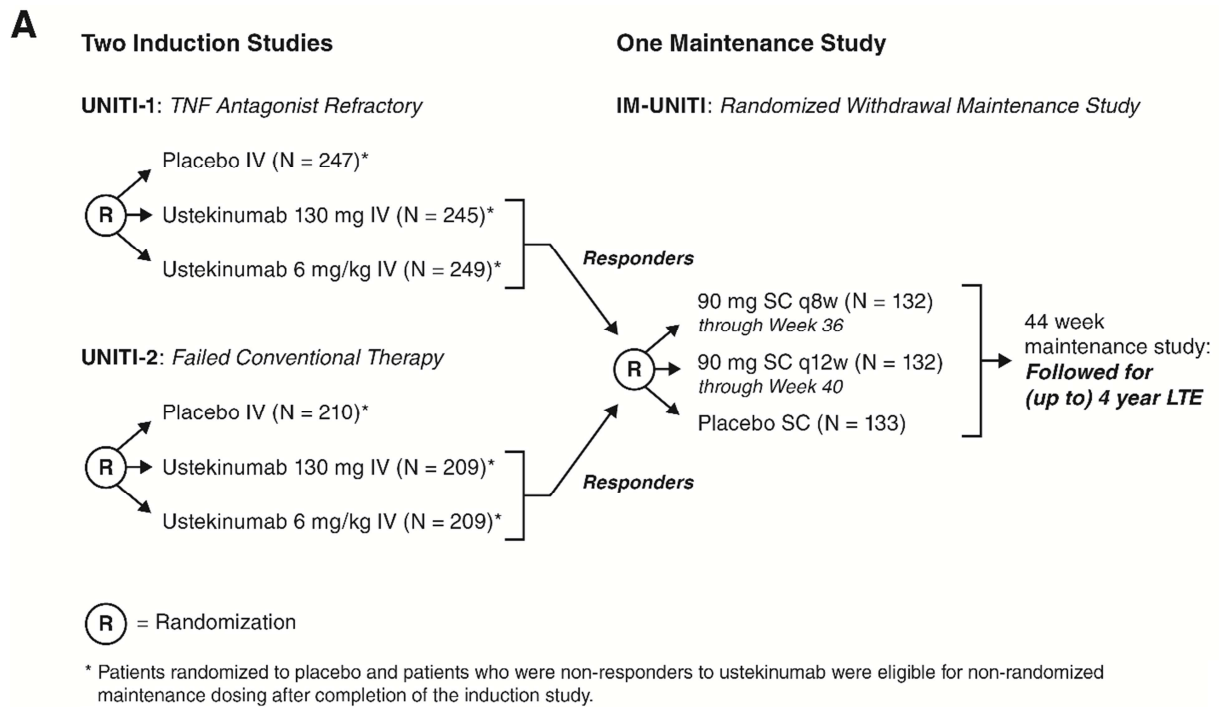
Supplemental Figure 1. Patient disposition throughout the UNITI-1, UNITI-2 and IM-UNITI studies among the 1366 patients who had serum ustekinumab concentration and efficacy outcome data suitable for analysis. ER, exposure-response; IV, intravenous; N, number of patients; PK, pharmacokinetic; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous.



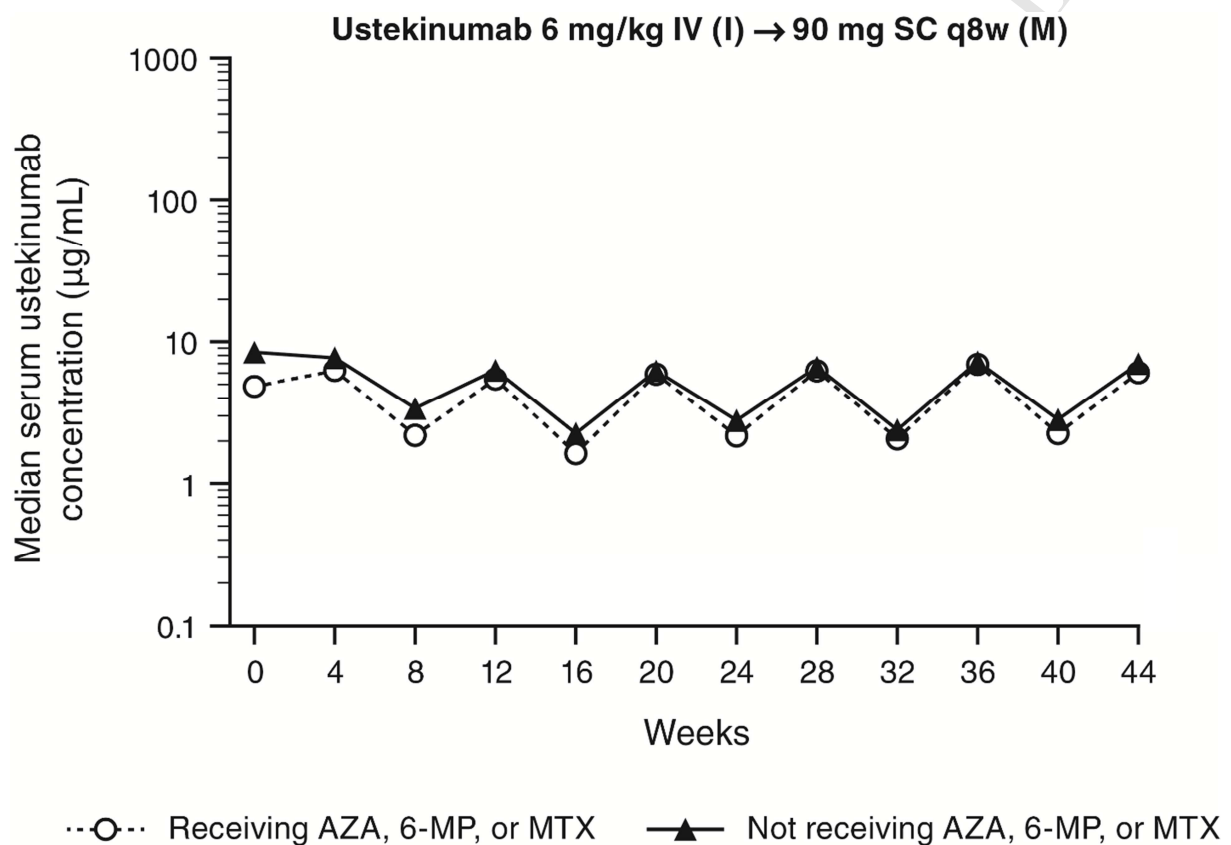
Supplemental Figure 2. Study design and flow for the UNITI-1, UNITI-2 and IM-UNITI studies (A) and in randomized and nonrandomized patients in IM-UNITI (B).

IV, intravenous; LTE, long-term extension; N, number of patients; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous; TNF, tumor necrosis factor.

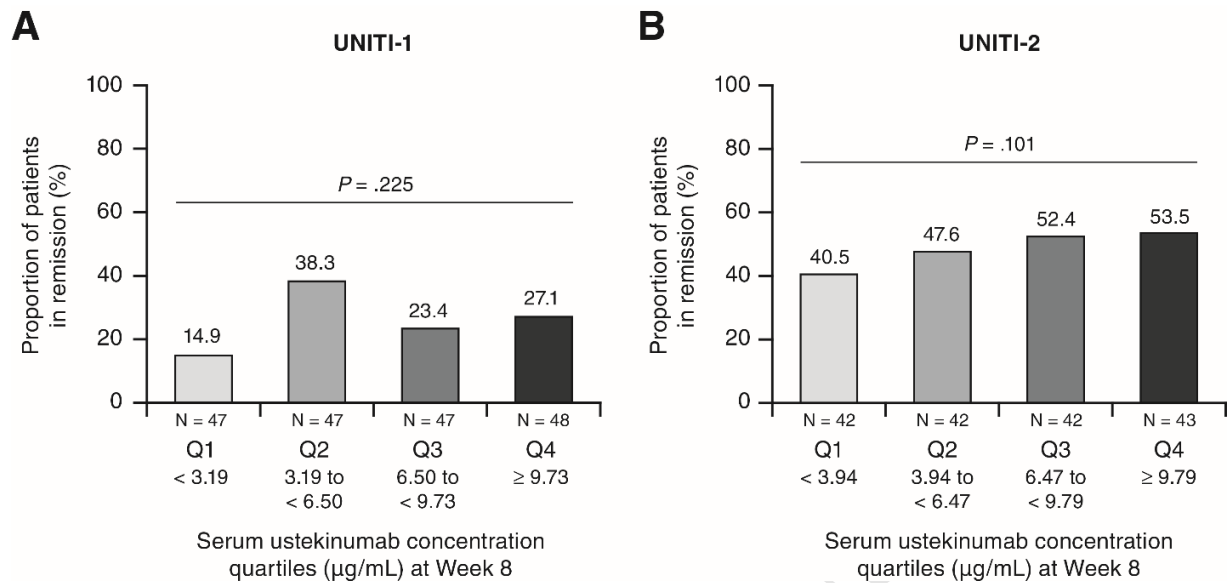
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Supplemental Figure 3. Median serum ustekinumab concentrations over time through the maintenance study IM-UNITI in those receiving or not receiving concomitant immunomodulators. 6-MP, 6-mercaptopurine; AZA, azathioprine; I, induction; IV, intravenous; M, maintenance; MTX, methotrexate; q8w, every 8 weeks; SC, subcutaneous.

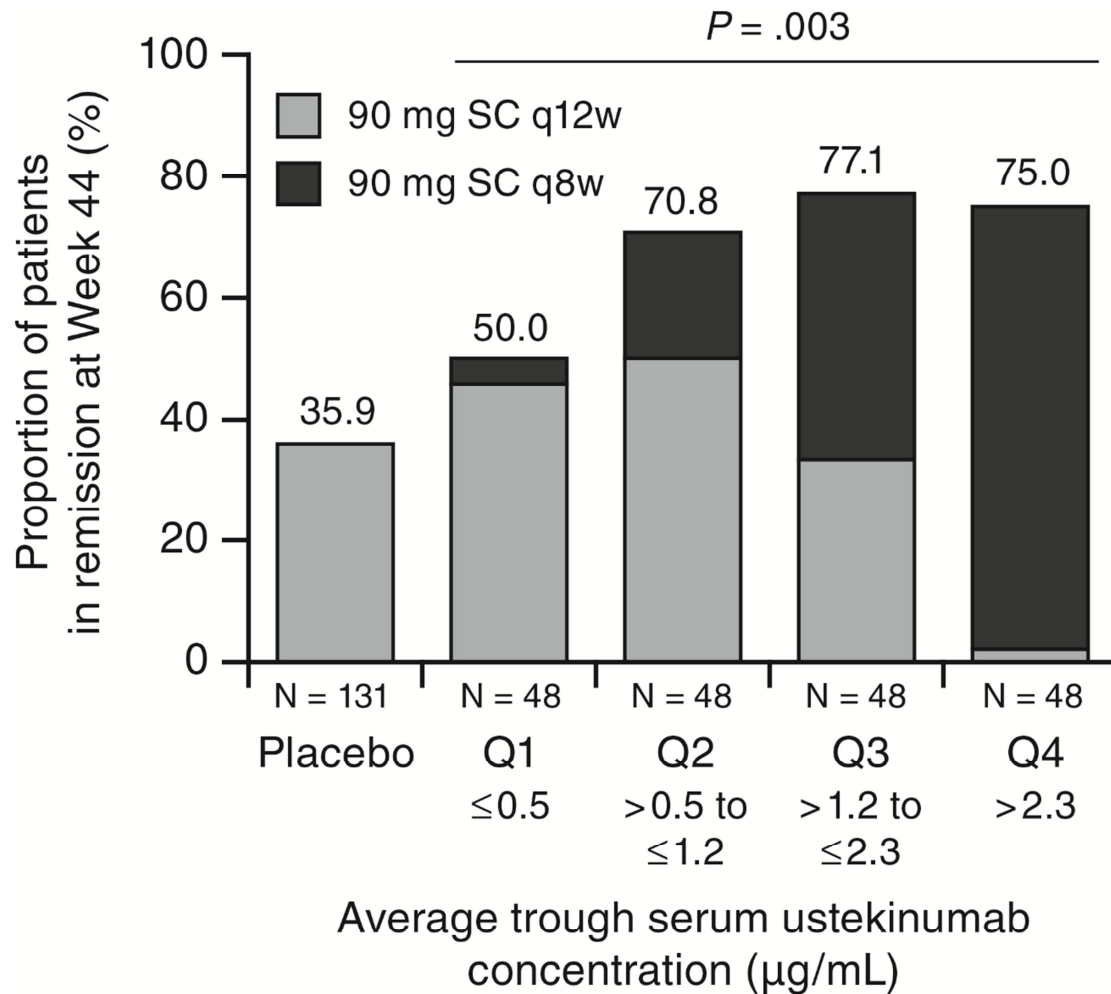


Supplemental Figure 4. Proportions of patients achieving clinical remission by serum ustekinumab concentration quartiles at Week 8 in the ~6 mg/kg dose in the UNITI-1 (A) and UNITI-2 (B) induction studies. N, number of patients; Q, quartile.



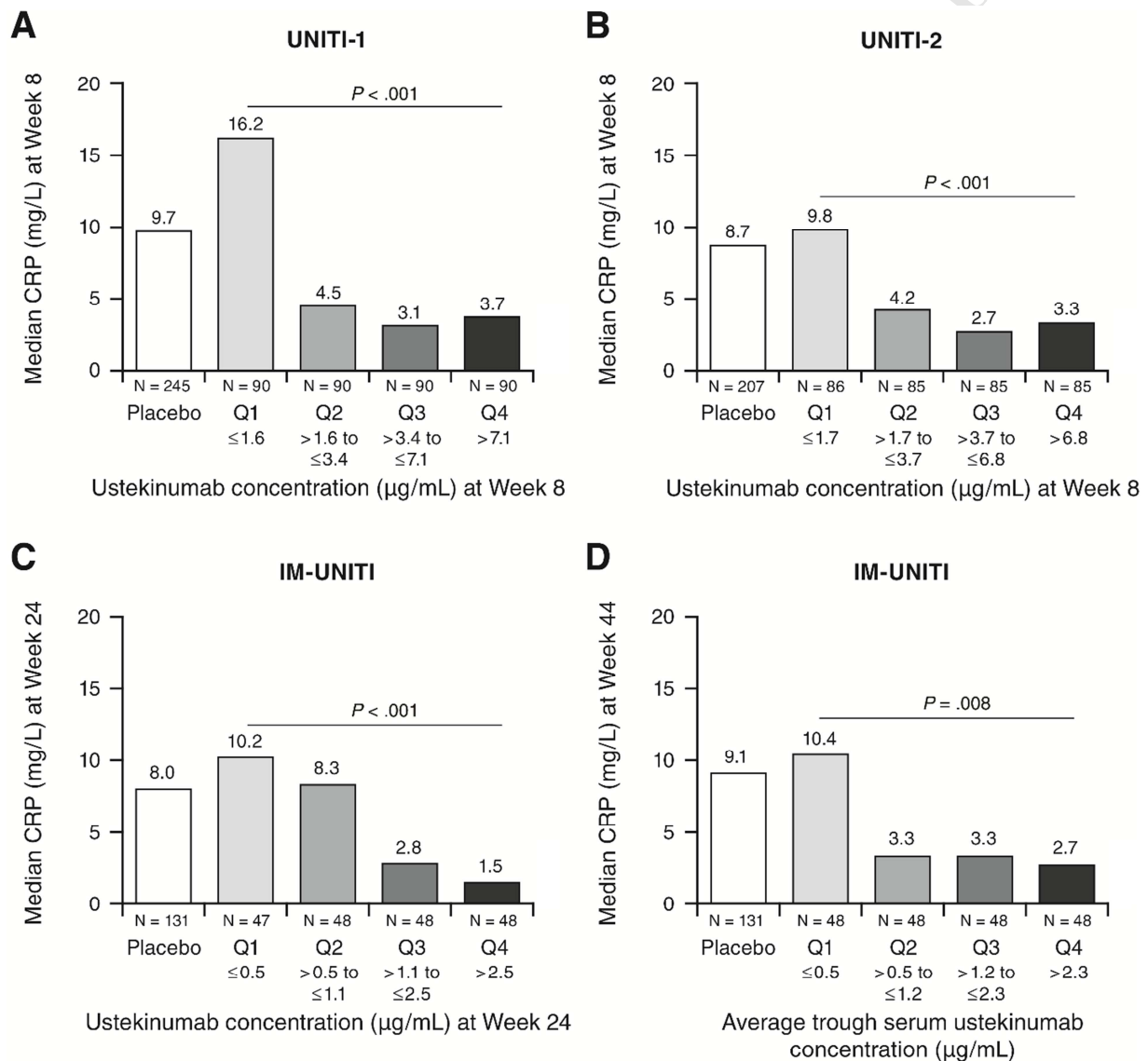
Supplemental Figure 5. Proportions of patients achieving clinical remission by average trough serum ustekinumab concentration quartiles at Week 44 in the IM-UNITI maintenance study. Patients in the placebo group received an IV induction dose of ustekinumab only and were on placebo treatment during maintenance. Average trough concentrations were obtained by computing the arithmetic mean of the observed trough concentration for each patient (q12w: Week 24, Week 36; q8w: Week 24, Week 32, and Week 40) to reflect average exposure at steady state. IV, intravenous; N, number of

patients; Q, quartile; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous.

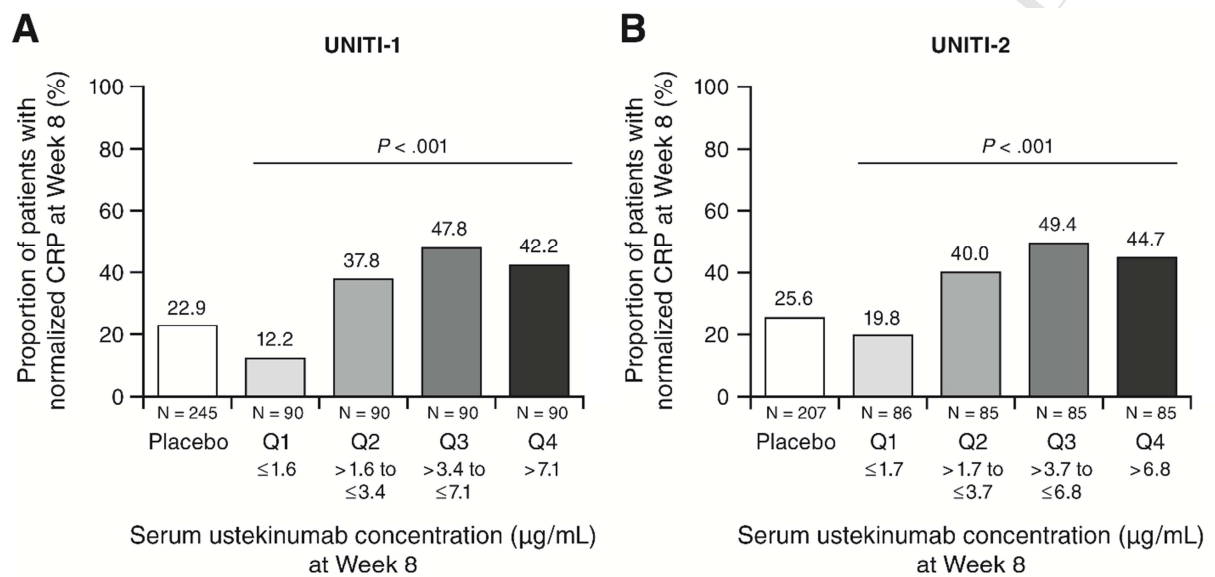


Supplemental Figure 6. Median C-reactive protein levels at Week 8 in the UNITI-1 (A) and UNITI-2 (B) induction studies and at Week 24 (C) and Week 44 (D) in the IM-UNITI maintenance study by serum ustekinumab concentration quartiles. In A and B, the quartiles represent the combined 130 mg and ~6 mg/kg induction treatments, and in C and D the quartiles represent the combined 90 mg SC q12w and q8w maintenance treatments. Patients in the placebo groups in C and D received an IV induction dose of ustekinumab only and were on placebo treatment during maintenance. Average trough

concentrations were obtained by computing the arithmetic mean of the observed trough concentration for each patient (q12w: Week 24, Week 36; q8w: Week 24, Week 32, and Week 40) to reflect average exposure at steady state. CRP, C-reactive protein; IV, intravenous; N, number of patients; Q, quartile; q8w, every 8 weeks; q12w, every 12 weeks; SC, subcutaneous.



Supplemental Figure 7. Proportions of patients achieving normalized C-reactive protein by serum ustekinumab concentration quartiles at Week 8 in the UNITI-1 (A) and UNITI-2 (B) induction studies. The quartiles represent the combined 130 mg and ~6 mg/kg induction treatments. CRP, C-reactive protein; N, number of patients; Q, quartile.



Supplemental Tables

Supplemental Table 1: Pharmacokinetic Sampling Schematic for the UNITI-1, UNITI-2, and IM-UNITI Studies.

Study	0	3	6	8	12	16	20	24	28	32	36	40	44	48	52
	(I-0)	(I-3)	(I-6)	(I-8/ M-0)	(M-4)	(M-8)	(M-12)	(M-16)	(M-20)	(M-24)	(M-28)	(M-32)	(M-36)	(M-40)	(M-44)
UNITI-1	● ^a	●	●	● ^b						NA					
UNITI-2	● ^a	●	●	● ^b						NA					
IM-UNITI		NA		● ^{b,c}	●	●	●	●	●	●	●	●	●	●	●

^aSamples obtained both pre-administration and at 1 hour post-infusion.

^bSample at Week 0 of maintenance (M-0) is the same as sample at Week 8 of induction (I-8).

^cPatients who were nonresponders to placebo during an induction study (UNITI-1/UNITI-2) received IV ustekinumab 130 mg at M-0 (IM-UNITI) and had pre- and post-infusion samples at M-0.

I, Induction; IV; intravenous; M, Maintenance; NA, Not applicable.

Supplemental Table 2: Baseline Patient Characteristics of the Patients in the UNITI-1, UNITI-2, and IM-UNITI Studies Included in the Pharmacokinetic/ Exposure-Response Analysis (N=1366).

Characteristic	Proportion
Male gender	44.6%
Concomitant azathioprine/6-mercaptopurine/methotrexate	33.1%
Concomitant corticosteroid use	34.6%
Prior TNF antagonist use	68.5%
Characteristic	Median (Range)
Age, yrs	37.0 (18.0 – 77.0)
Body weight, kg	68.8 (35.0 – 184.0)
Disease duration, yrs	8.7 (0.1 – 52.4)
CDAI ^a	305.0 (198.0 – 515.0)
CRP, mg/L	9.1 (0.1 – 18.0)
Fecal calprotectin, µg/kg	483.0 (11.8 – 16647.2)
Fecal lactoferrin, µg/mL	90.9 (0.4 – 1002.3)
Serum albumin, g/dL	3.6 (1.2 – 5.1)

^aCDAI scores range from approximately 0 to 600, with higher scores indicating worse disease and a 50-point change indicating the minimal clinically important difference.

CDAI, Crohn's Disease Activity Index; CRP, C-reactive protein; N, number of patients; TNF, tumor necrosis factor; Yrs, years.

Supplemental Table 3: Ustekinumab Concentrations and Selected Safety Events During the UNITI-1, UNITI-2, and IM-UNITI Studies.

Safety Event	Induction: UNITI-1 and UNITI-2				
	Incidence through Week 8				
	Placebo (N=452)	Q1 (N=175)	Q2 (N=175)	Q3 (N=176)	Q4 (N=175)
Infections, (%)	23.5	21.1	21.7	22.7	20.6
Serious Infections, (%)	1.3	3.4	0.0	1.7	0.6
SAEs, (%)	6.0	8.0	4.6	2.8	1.7

Serum ustekinumab concentration at Week 8 was used as the systemic exposure metric; Q1: ≤ 1.6 µg/mL, Q2: >1.6 to ≤ 3.5 µg/mL, Q3: >3.5 to ≤ 6.8 µg/mL, Q4: >6.8 µg/mL

Maintenance: IM-UNITI

Safety Event	Incidence through Week 44				
	Placebo (N=131)	Q1 (N=48)	Q2 (N=48)	Q3 (N=48)	Q4 (N=48)
Infections, (%)	67.2	58.3	43.8	66.7	58.3
Serious Infections, (%)	3.1	8.3	2.1	2.1	4.2
SAEs, (%)	21.4	12.5	14.6	4.2	12.5

Average steady-state serum ustekinumab concentration was used as the systemic exposure metric;

Q1: $\leq 0.5 \mu\text{g/mL}$, Q2: >0.5 to $\leq 1.1 \mu\text{g/mL}$, Q3: >1.1 to $\leq 2.3 \mu\text{g/mL}$, Q4: $>2.3 \mu\text{g/mL}$

N, number of patients; Q, quartile; SAE, serious adverse event.

