Higher Drug Exposure During the First 24 Weeks of Ustekinumab Treatment Is Associated With Endoscopic Remission in Crohn's Disease

Over the past 2 decades, the treatment of Crohn's disease (CD) has significantly changed with the introduction of biologicals targeting key inflammatory players. Ustekinumab is a human monoclonal antibody directed against the common p40 subunit of interleukin-12 and interleukin-23, cytokines involved in the immune cascade of chronic immune-mediated inflammatory diseases.[1] As with all biologicals, nonresponse or loss of response to treatment can occur when treating patients with CD with ustekinumab. The UNITI registration trials and subsequent real-world studies reported clinical response rates of around 35%–55% after ustekinumab induction therapy.[2,3] Inadequate response to treatment can be associated with underexposure to the drug. Several studies have demonstrated the relationship between ustekinumab concentrations and clinical, biologic, or endoscopic response, indicating the potential usefulness of therapeutic drug monitoring to guide clinical decision-making.[4–6] Anti–tumor necrosis factor drug concentrations measured during induction are associated with long-term outcomes.[7,8] For ustekinumab, however, the time point at which the drug concentration is most informative for long-term outcome is unknown. In this study, we evaluated the exposure-response relationship of ustekinumab throughout the first 24 weeks of treatment in patients with CD and investigated the time points at which ustekinumab levels could identify patients achieving endoscopic remission.

A total of 19 patients who started ustekinumab therapy for active CD all received an intravenous infusion of ~6 mg/kg ustekinumab followed by subcutaneous dosing of 90 mg every 8 weeks. The primary outcome was endoscopic remission defined as Simple Endoscopic Score for CD (SES-CD) ≤3 after 6 months of therapy. To facilitate multiple sampling, a validated (Supplementary Methods) method of dried blood spot (DBS) sampling was applied for the measurement of ustekinumab concentrations. DBS sampling refers to the collection of blood on a protein saver card through a small finger prick, from which the drug can be extracted and measured. Samples were collected at 23 time points over the first 24 weeks of ustekinumab therapy (Week 1, Week 3, Week 4, Week 6, Week 8, Week 8 + 1 day, Week 8 + 3 days, Week 8 + 5 days, Week 9, Week 9 + 2 days, Week 10, Week 11, Week 12, Week 16, Week 16 + 1 day, Week 16 + 3 days, Week 16 + 5 days, Week 17, Week 17 + 2 days, Week 18, Week 19, Week 20, and Week 24) and ustekinumab concentrations were measured by enzyme-linked immunosorbent assay (CE-labeled Ustekinumab ELISA kit, apDia, Turnhout, Belgium). The local ethics committee approved the study and all patients provided written informed consent (S62619/EudraCT-nr: 2019-002038-35).

Spiking various ustekinumab concentrations (0.2–80 μg/mL) in citrated whole blood and subsequent spotting and extraction showed a consistent ustekinumab recovery and a coefficient of variation <18%. For each spiked concentration, the measured ustekinumab concentration from spotted blood volumes between 15 and 50 μL was similar. Storing the DBS cards at room temperature for up to 2 weeks and the DBS extracts for up to 2 months at −20°C did not affect the ustekinumab recovery (data not shown).

Of the 19 ustekinumab-treated patients with CD included in this study (Supplementary Table 1), 4 (21%) achieved endoscopic remission. DBS sampling at trough and at various intermediate time points allowed construction of a ustekinumab concentration-time profile, which showed a small peak after each subcutaneous ustekinumab injection that would not be captured when only sampling at trough would be performed (Figure 1*A*). High variability was observed between the individual concentration-time profiles of the 19 ustekinumab-treated patients with CD. Median concentration-time profiles showed that patients in remission (n = 4) had a significantly higher median area under the curve (AUC) from baseline to Week 24, hence a higher drug exposure, than patients not achieving remission (n = 15) (897 vs 479 μg*day/mL; $P < .005$; Figure 1*B*). A similar observation could be made for the AUC from baseline to Week 8 (517 vs 275 μg*day/mL; $P < .01$) and from baseline to Week 16 (743 vs 404 μg*day/mL; $P < .005$) but not for the AUC from baseline to Week 4 (304 vs 209 μg*day/mL; $P = .0624$). Moreover, a negative correlation was observed between the AUC and SES-CD at Week 24 (n = 19; Spearman $r = -0.69$; $P < .002$; data not shown).

At multiple time points, the ustekinumab concentration in DBS samples was significantly different between

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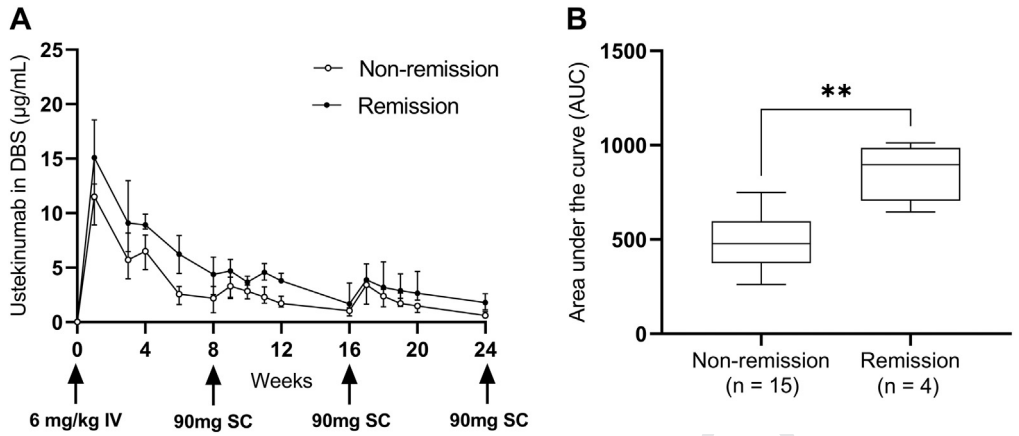

Figure 1. Ustekinumab concentration-time profiles (*A*) and area under the curve (*B*) of patients in endoscopic remission and nonremission. (*A*) Median ustekinumab concentrations (with interquartile range) relative to the number of weeks on ustekinumab therapy in patients in endoscopic remission (*black circles*) compared with patients without endoscopic remission (*white circles*). *Arrows* indicate the time point and dose of administered ustekinumab. (*B*) Median area under the curve (AUC) from baseline to Week 24 of ustekinumab therapy in remitters (n = 4; 897 µg*day/mL) and nonremitters (n = 15; 479 µg*day/mL). Mann-Whitney *U* test. $^{**}P < .005$; IV, intravenous; SC, subcutaneous.

patients achieving endoscopic remission and patients not achieving remission (Supplementary Table 2). Ustekinumab concentrations were significantly higher in patients achieving endoscopic remission compared with patients not achieving this outcome, at trough and all evaluated intermediate time points except at Week 1 and, strikingly, also not at the 2 weeks after the subcutaneous dosing at multiple time points (ie, Week 8 + 1 day, Week 9, Week 9 + 2 days, Week 10, Week 16 + 1 day, Week 17, and Week 18). When evaluating endoscopic response, defined as a 50% reduction in SES-CD, ustekinumab concentrations were also significantly different between responders and nonresponders but at less time points than when remission was the evaluated outcome (Supplementary Table 2). A possible explanation for this observation is that the responder's group is a mix of patients with residual inflammation who might relapse and of patients that ultimately achieve endoscopic remission.

To the best of our knowledge, only 1 study thus far has investigated ustekinumab exposure through the AUC and the relationship with treatment response. In that study by Hanzel et al,[9] serum samples from 41 ustekinumab-treated patients with CD were collected at 1 hour after the intravenous infusion, at Weeks 2, 4, and 8 of treatment. They observed that biochemical, but not endoscopic, remission was associated with ustekinumab exposure over the first 8 weeks of treatment.[9]

The presence of a concentration-response relationship at trough for ustekinumab in CD is confirmed in our study.[4–6,9,10] Ustekinumab concentrations at time points between 2 ustekinumab administrations, however, had not yet been extensively investigated. Interestingly, ustekinumab levels were not significantly higher in endoscopic remitters compared with nonremitters at time points immediately following subcutaneous dosing. This could suggest that not the absorption but rather the clearance of ustekinumab may be different between the remitters and nonremitters. Alternatively, significance might not be reached because of the small sample size. Overall, these findings indicate that the first 2 weeks after the subcutaneous ustekinumab injections are not optimal time points to monitor ustekinumab concentrations. Because the concentration-response relationship was observed at multiple time points and no single time point in particular is standing out, monitoring ustekinumab levels can be useful at various time points. Feasibility should also be taken into account when deciding on the time point to monitor ustekinumab concentrations. Larger prospective trials are needed to identify the ustekinumab concentration threshold associated with endoscopic remission at 1 or more of the time points identified in this study.

In conclusion, patients with CD achieving endoscopic remission at Week 24 of ustekinumab therapy have a higher ustekinumab drug exposure than patients not achieving endoscopic remission. Monitoring ustekinumab concentrations at trough or at intermediate time points could help to timely identify patients achieving endoscopic (non)-remission. Q10

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2012.11.00.

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Supplementary Methods

For the analytical validation of the DBS method, ustekinumab concentrations (0.2–80 μg/mL) were spiked in citrated whole blood of healthy donors (Valley Biomedical, Winchester, VA) and spotted (40 μL) onto a filter paper (Whatman 903 Protein Saver Card, GEHealthcare, Diegem, Belgium). After air-drying the card overnight, a 6-mm diameter disc completely impregnated with blood was punched out. The drug was extracted from the disc by addition of 240 μL Super-BlockT20 buffer (Thermo Fisher Scientific, Rockford, IL) and shaking at 21°C for 1 hour. Ustekinumab concentrations were subsequently measured using an in-house enzyme-linked immunosorbent assay with antibody MA-UST56A2D11 for capture and MA-UST56C1H12 for detection.

The impact of the spotted volume of blood was investigated by spiking ustekinumab (0.2–80 μg/mL) to citrated whole blood and spotting different volumes (15–50 μL) onto filter paper. Subsequently, ustekinumab was measured as previously described.

The stability of ustekinumab in DBS cards kept at room temperature and in DBS extracts at −20°C was investigated by spotting different concentrations of ustekinumab (0.2–80 μg/mL) on different filter papers. The ustekinumab recovery after storing the DBS card at room temperature for 1 week, 2 weeks, and 4 weeks (relative to overnight drying) or the DBS extracts at −20°C for 1 or 2 months (relative to before freezing), was evaluated.

Supplementary Table 1. Baseline Characteristics of the 19 Included Crohn's Disease Patients

	Remitters	Nonremitters
Number of patients, n (%)	4 (21)	15 (79)
Sex, women, n (%)	3 (75)	6 (40)
Age, median (IQR), *y*	40.9 (33.5–53.2)	40.8 (32.4–51.3)
Disease duration, median (IQR), *y*	16.3 (12.7–25.6)	16.6 (7.4–26.7)
Simple Endoscopic Score for Crohn's disease, median (IQR)	8.0 (6.5–10.0)	12.0 (7.5–15.0)
Body mass index, kg/m^2, median (IQR)	23.9 (22.6–24.7)	23.3 (22.6–25.8)
Previous biologic therapy, n (%)		
Anti-TNF	3 (75)	12 (80)
Vedolizumab	1 (25)	5 (33)
Concomitant steroids, n (%)	0	3 (20)
Disease location, n (%)		
Ileal disease (L1)	1 (25)	5 (33)
Colonic disease (L2)	1 (25)	1 (7)
Ileocolonic disease (L3)	2 (50)	9 (60)
Upper GI involvement (L4)	0	2 (13)
Disease behavior, n (%)		
Inflammatory (B1)	2 (50)	6 (40)
Stricturing (B2)	1 (25)	6 (40)
Penetrating (B3)	1 (25)	3 (20)
Perianal disease (p)	1 (25)	4 (27)
Smoking status, n (%)		
Active smoking	0	3 (20)
Previously smoking	0	2 (13)
Never smoked	4 (100)	10 (67)

GI, gastrointestinal; IQR, interquartile range; TNF, tumor necrosis factor.

Supplementary Table 2. Ustekinumab Concentration-Response Analyses

Time point	UST concentration (μg/mL)				UST concentration (μg/mL)			
	Nonremission (n = 15)	Remission (n = 4)	*P* value[a]	AUROC	Nonresponse (n = 12)	Response (n = 7)	*P* value[a]	AUROC
Week 1	11.5 (9.4–12.6)	15.1 (12.5–17.6)	NS	0.78	11.5 (9.7–12.6)	13.1 (11.3–15.7)	NS	0.70
Week 3	5.7 (4.0–7.8)[b]	9.1 (8.0–10.6)	< .05	0.84	6.0 (4.1–8.2)	7.3 (5.8–9.3)[b]	NS	0.67
Week 4	6.4 (4.7–7.6)[b]	8.9 (8.6–9.5)	< .02	0.89	6.9 (5.7–8.0)[b]	8.5 (4.8–8.9)	NS	0.59
Week 6	2.6 (1.6–3.3)	6.2 (5.6–6.9)	< .005	0.95	2.8 (2.1–3.6)	3.9 (1.7–6.2)	NS	0.62
Week 8[c]	2.2 (1.2–2.9)	4.4 (3.4–5.3)	< .05	0.85	2.3 (1.3–3.3)	2.2 (2.1–4.4)	NS	0.63
Week 8 + 1 d	2.4 (2.1–3.9)[d]	4.4 (4.2–4.6)	NS	0.82	2.4 (1.9–3.5)[e]	4.3 (4.2–4.5)[e]	NS	0.82
Week 8 + 3 d	2.8 (2.3–3.8)[f]	4.4 (4.2–5.3)	< .05	0.85	2.7 (2.2–3.4)[b]	4.5 (4.3–6.7)[e]	< .01	0.93
Week 8 + 5 d	3.5 (2.1–4.0)[e]	5.9 (5.3–6.6)	< .005	0.96	3.5 (1.9–4.0)[b]	5.0 (4.0–6.2)[b]	< .05	0.83
Week 9	3.3 (2.3–3.9)	4.7 (3.6–5.4)	NS	0.72	3.0 (2.1–4.2)	3.7 (3.0–4.7)	NS	0.64
Week 9 + 2 d	2.9 (2.5–4.3)[d]	5.3 (4.1–6.0)	NS	0.73	3.2 (2.3–4.4)[e]	4.9 (3.0–5.8)[e]	NS	0.68
Week 10	2.8 (2.2–3.4)	3.7 (3.4–4.0)	NS	0.78	2.6 (2.0–3.4)	3.5 (3.1–3.7)	NS	0.75
Week 11	2.3 (1.8–3.2)	4.6 (4.0–5.2)	< .001	1.00	2.1 (1.7–3.2)	3.8 (3.2–4.6)	< .01	0.86
Week 12	1.7 (1.5–2.4)	3.8 (3.8–4.0)	< .001	1.00	1.6 (1.4–2.4)	3.8 (2.2–3.8)	< .02	0.85
Week 16[c]	1.1 (0.6–1.2)	1.7 (1.4–2.4)	< .02	0.88	1.1 (0.6–1.3)	1.3 (1.0–1.7)	NS	0.70
Week 16 + 1 d	1.9 (1.5–2.4)	2.4 (2.3–2.8)	NS	0.77	1.8 (1.2–2.2)	2.4 (2.3–2.7)	< .01	0.87
Week 16 + 3 d	2.5 (2.3–2.7)	3.4 (3.1–4.0)	< .02	0.88	2.4 (2.0–2.5)	3.4 (3.1–3.7)	< .001	0.94
Week 16 + 5 d	2.9 (2.2–3.1)[b]	4.1 (3.9–4.7)	< .01	0.93	2.9 (1.9–3.0)[b]	4.1 (3.3–4.4)	< .01	0.88
Week 17	3.4 (2.0–3.8)	3.9 (3.5–4.6)	NS	0.77	2.9 (1.6–3.8)	3.5 (3.5–4.0)	NS	0.71
Week 17 + 2 d	3.0 (1.9–3.3)[e]	4.1 (4.0–4.7)	< .005	0.96	3.1 (1.7–3.4)[b]	3.9 (3.4–4.2)[b]	< .05	0.82
Week 18	2.4 (1.7–2.9)	3.2 (2.8–4.2)	NS	0.82	2.3 (1.4–2.6)	3.0 (2.7–3.3)	< .05	0.79
Week 19	1.7 (1.5–2.4)	2.9 (2.6–3.5)	< .02	0.88	1.7 (1.4–2.3)	2.7 (2.0–2.9)	NS	0.76
Week 20	1.5 (1.1–1.7)	2.7 (2.3–3.7)[b]	< .01	0.96	1.4 (0.9–1.7)	2.3 (1.8–2.6)[b]	< .01	0.88
Week 24[c]	0.6 (0.5–0.9)[d]	1.8 (1.5–2.1)	< .02	0.93	0.6 (0.5–0.7)[d]	1.1 (1.0–1.8)	< .01	0.89

NOTE. No correction for multiple testing was performed.
AUROC, area under the receiving operating characteristics curve; DBS, dried blood spot; NS, not significant; UST, ustekinumab.
[a]Mann-Whitney *U* test for comparing ustekinumab concentrations of remitters versus nonremitters and responders versus nonresponders. Ustekinumab concentrations are represented as median (interquartile range).
[b]1 datapoint missing.
[c]Trough.
[d]4 datapoints missing.
[e]2 datapoints missing.
[f]3 datapoints missing.