CLINICAL STUDY REPORT

DUST study: a prospective study investigating ustekinumab concentrations through dried blood spot sampling in Crohn's disease patients (S62619, Eudra-CT 2019-002038-35)

Since their introduction in the nineties, biologicals have revolutionized the care and outlook of patients living with inflammatory bowel diseases (IBD). The efficacy of biologicals, however, shows high intra- and inter-individual variability: some patients do not respond at all while other initially respond but lose response over time, and again others enter into a deep and prolonged remission. Non-response or loss of response is often the result of sub-therapeutic drug concentrations. Therefore, therapeutic drug monitoring (TDM) has been introduced in order to measure drug concentrations and optimize treatment when necessary.

Several studies have demonstrated the relationship between ustekinumab trough concentration and clinical, biological and endoscopic response indicating the usefulness of therapeutic drug monitoring to guide clinical decision-making. It is however still unknown at which time point one should measure ustekinumab concentrations and which ustekinumab concentration one should target to obtain meaningful outcomes.

OBJECTIVE

To investigate the ustekinumab concentrations at multiple time points throughout the first 24 weeks of ustekinumab therapy in Crohn's disease (CD) patients through intensive blood sampling to identify the best time point to measure ustekinumab during induction to predict long-term outcome.

INCLUSION

Between July 2019 and April 2021, a total of **22 CD patients** who start ustekinumab treatment were included in the study. Informed consent was signed before inclusion. Patients performed dried blood spot (DBS) sampling at several time points at home (w1, w3, w4, w6, w8, w8+1d, w8+3d, w8+5d, w9, w9+2d, w10, w11, w12, w16, w16+1d, w16+3d, w16+5d, w17, w17+2d, w18, w19, w20, w24). Patients came to the hospital for clinical evaluation, venous serum sampling and ustekinumab administration following routine clinical practice. No extra visits to the hospitals were necessary.

MEASUREMENTS

Demographic information and disease characteristics were obtained including sex, age at diagnosis, height, body weight, body mass index, disease location, disease behaviour (including perianal disease), smoking status (never, ex, active), current and previous use of medication. Clinical disease activity was assessed using the Harvey-Bradshaw index (HBI) and endoscopic disease activity using the Simple Endoscopic Score for Crohn's disease (SES-CD). Baseline characteristics of the patients are shown in Table 1.

Table 1

	Remitters	**Non-remitters**
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/m2, median (IQR)	23.9 (22.6-24.7)	23.3 (22.6-25.8)
Previous biological 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 behaviour, 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%)

* remission is defined as simple endoscopic score for CD (SES-CD) ≤3 after 6 months of therapy.

Ustekinumab concentrations in patient serum and DBS extract was determined with an in-house developed sandwich-type enzyme-linked immunosorbent assay (ELISA).

ANALYSES

Interim analysis was performed on a total of 19 patients. 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 1A). High variability was observed between the individual concentration-time profiles of the 19 ustekinumab-treated CD patients. 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<0.005, Figure 1B). A similar observation could be made for the AUC from baseline to week 8 (517 vs 275 µg*day/mL, p <0.01) and from baseline to week 16 (743 vs 404 µg*day/mL, p<0.005) but not for the AUC from baseline to week 4 (304 vs 209 µg*day/mL, p = 0.0624). Moreover, a negative correlation was observed between the AUC and SES-CD at week 24 (n= 19, Spearman r = -0.69, p<0.002, data not shown).

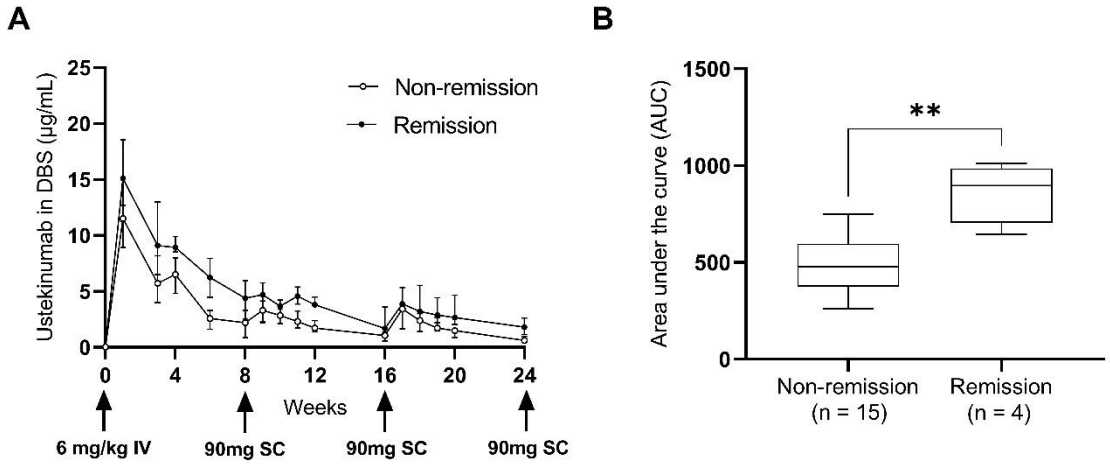

Figure 1 Ustekinumab concentration-time profiles (A) and area under the curve (B) of patients in endoscopic remission and non-remission. Fig 1A shows the median ustekinumab concentrations (with interquartile range) relative to the number of weeks on ustekinumab therapy in patients in endoscopic remission (closed circles) compared to patients without endoscopic remission (open circles). Arrows indicate the time point and dose of administered ustekinumab. Fig 1B gives the median area under the curve (AUC) from baseline to week 24 of ustekinumab therapy in remitters (n = 4, 897 µg*day/mL) and non-remitters (n = 15, 479 µg*day/mL). Mann-Whitney U-test. **p < 0.005; DBS, dried blood spot; IV, intravenous; SC, subcutaneous

At multiple time points, the ustekinumab concentration in DBS samples was significantly different between patients achieving endoscopic remission and patients not achieving remission (Table 2). Ustekinumab concentrations were significantly higher in patients achieving endoscopic remission compared to patients not achieving this outcome, at trough and all evaluated intermediate time points except at week 1 and, strikingly, also not at the two weeks after the subcutaneous dosing at multiple time points (i.e., week 8+1d, week 9, week 9+2d, week 10, week 16+1d, week 17, and week 18). When evaluating endoscopic response, defined as a 50% reduction in SES-CD score, ustekinumab concentrations were also significantly different between responders and non-responders but at less time points then when remission was the evaluated outcome (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.

Table 2

Time point	UST concentration (µg/mL)		p-value*	AUROC	UST concentration (µg/mL)		p-value*	AUROC
	Non-remission (n = 15)	*Remission (n = 4)*			*Non-response (n = 12)*	*Response (n = 7)*		
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)[A]	9.1 (8.0-10.6)	<0.05	0.84	6.0 (4.1-8.2)	7.3 (5.8-9.3)[A]	NS	0.67
Week 4	6.4 (4.7-7.6)[A]	8.9 (8.6-9.5)	<0.02	0.89	6.9 (5.7-8.0)[A]	8.5 (4.8-8.9)	NS	0.59
Week 6	2.6 (1.6-3.3)	6.2 (5.6-6.9)	<0.005	0.95	2.8 (2.1-3.6)	3.9 (1.7-6.2)	NS	0.62
Week 8[‡]	2.2 (1.2-2.9)	4.4 (3.4-5.3)	<0.05	0.85	2.3 (1.3-3.3)	2.2 (2.1-4.4)	NS	0.63
Week 8 + 1d	2.4 (2.1-3.9)[D]	4.4 (4.2-4.6)	Ns	0.82	2.4 (1.9-3.5)[B]	4.3 (4.2-4.5)[B]	NS	0.82
Week 8 + 3d	2.8 (2.3-3.8)[C]	4.4 (4.2-5.3)	<0.05	0.85	2.7 (2.2-3.4)[A]	4.5 (4.3-6.7)[B]	<0.01	0.93
Week 8 + 5d	3.5 (2.1-4.0)[B]	5.9 (5.3-6.6)	<0.005	0.96	3.5 (1.9-4.0)[A]	5.0 (4.0-6.2)[A]	<0.05	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 + 2d	2.9 (2.5-4.3)[D]	5.3 (4.1-6.0)	Ns	0.73	3.2 (2.3-4.4)[B]	4.9 (3.0-5.8)[B]	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)	<0.001	1.00	2.1 (1.7-3.2)	3.8 (3.2-4.6)	<0.01	0.86
Week 12	1.7 (1.5-2.4)	3.8 (3.8-4.0)	<0.001	1.00	1.6 (1.4-2.4)	3.8 (2.2-3.8)	<0.02	0.85
Week 16[‡]	1.1 (0.6-1.2)	1.7 (1.4-2.4)	<0.02	0.88	1.1 (0.6-1.3)	1.3 (1.0-1.7)	NS	0.70
Week 16 + 1d	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)	<0.01	0.87
Week 16 + 3d	2.5 (2.3-2.7)	3.4 (3.1-4.0)	<0.02	0.88	2.4 (2.0-2.5)	3.4 (3.1-3.7)	<0.001	0.94
Week 16 + 5d	2.9 (2.2-3.1)[A]	4.1 (3.9-4.7)	<0.01	0.93	2.9 (1.9-3.0)[A]	4.1 (3.3-4.4)	<0.01	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 + 2d	3.0 (1.9-3.3)[B]	4.1 (4.0-4.7)	<0.005	0.96	3.1 (1.7-3.4)[A]	3.9 (3.4-4.2)[A]	<0.05	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)	<0.05	0.79
Week 19	1.7 (1.5-2.4)	2.9 (2.6-3.5)	<0.02	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)[A]	<0.01	0.96	1.4 (0.9-1.7)	2.3 (1.8-2.6)[A]	<0.01	0.88
Week 24[‡]	0.6 (0.5-0.9)[D]	1.8 (1.5-2.1)	<0.02	0.93	0.6 (0.5-0.7)[D]	1.1 (1.0-1.8)	<0.01	0.89

No correction for multiple testing was performed. [A] one datapoint missing; [B] two datapoints missing; [C] three datapoints missing; [D] four datapoints missing; [‡] trough; *Mann-Whitney U test for comparing ustekinumab concentrations of remitters vs non-remitters and responders vs non-responders. Ustekinumab concentrations are represented as median (interquartile range). AUROC, area under the receiving operating characteristics curve; DBS, dried blood spot; UST, ustekinumab; d, day; ns, not significant

CONCLUSION

In conclusion, CD patients achieving endoscopic remission at week 24 of ustekinumab therapy have a higher ustekinumab drug exposure than patients not achieving endoscopic remission. Because not one time point was superior to the other, monitoring ustekinumab concentrations at several trough or at intermediate time points could help to timely identify patients achieving endoscopic (non)-remission.

Because of these sufficient positive interim results, we only included 22 of the 30 intended CD patients.

TIMING

Inclusion of patients: July 2019 to April 2021

Analysis: 2021

Publication: manuscript provisionally accepted for publication in Clinical Gastroenterology and Hepatology (02/11/2021), minor revisions