Background: A fistula is an abnormal tract connecting two epithelialized surfaces, for example the intestine and the skin. Perianal fistula are a common complication of patients suffering Crohn's Disease (CD), but also occur in non-IBD patients in the form of cryptoglandular fistula. Around one third of all CD patients develop fistula at some point during their disease course. Fistula are often refractory to therapy, due to poor wound healing responses. In contrast, cryptoglandular fistula often respond to standard therapy. The biological background of this difference is unknown, and comparative studies between the two groups are lacking. The aim of this study was to characterize the cellular composition in fistula tracts of CD and cryptoglandular patients.
Methods: Curettage material of perianal fistula tracts was obtained during surgical intervention from patients with CD (n=15) and cryptoglandular fistulas (n=5). Single-cell suspensions were stained with a 35-antibody panel, focusing on myeloid and T-cell markers and were analyzed using mass cytometry (CyTOF). To visualize macrophages in the fistula tract we performed in situ hybridization with CD68 and TNF-α.
Results: The main cellular component of both fistula tracts consisted of CD66a+ granulocytes (64 +/- 24%). However, the remaining mononuclear compartment differed significantly between Crohn and cryptoglandular fistula. In CD, the majority was of lymphoid nature (CD3+ T cells 57 +/-21%, CD19+ B cells 14 +/-15%), while in cryptoglandular tracts, the majority consisted of myeloid origin (61+/- 15%). Within the T cell compartment, the majority of cells was CD45RO+, indicating activation. Presence of a seton increased the proportion of CD45RO+ T cells, in particular in CD4+ cells. In the myeloid compartment, CD14high/HLA-int monocytes, CD14int/HLA-high inflammatory macrophages and CD14high/CD163+ resident macrophages were identified. Interestingly, CD patient samples contained less monocyte-like cells, and substantially more resident macrophages compared to cryptoglandular samples. This feature tended to be even more enhanced in the presence of a seton, although this did not reach statistical significance. In situ hybridization showed a high production of TNF-α in epithelial-like cells in fistula tract of Crohn's disease patients, but not in macrophages.
Conclusion: Despite granulocytes being the main contributor to the cellular composition of fistula tracts, striking differences were found between Crohns and cryptoglandular fistula, both in lymphoid/myeloid balance, and in the presence of resident macrophages. We also showed that epithelial-like cells in Crohns's disease fistula tracts produce high amounts of TNF-α. These differences may contribute to the lack of response to therapy in CD.

DOP25

Association of Enterobacteriaceae with Crohn's Disease subtypes during remission

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Background: Members of the *Enterobacteriaceae* have been associated with active Crohn's Disease (CD), possibly as a result of intestinal inflammation via production of a lipopolysaccharide that can trigger TLR4 signalling. This study aims to assess whether this association persists in remission of CD patients and whether correlation with disease phenotype is present.
Methods: Stool samples of 32 CD patients in remission and 97 healthy controls were analyzed by 16S rRNA sequencing. High quality Amplicon sequence variants (ASV) were derived and classified via DADA2.
Results: ASV 6-*Escherichia/Shigella uncl.* was found to be more abundant in CD (padj=0.0003) while ASV 24, another member of the *Escherichia/Shigella* cluster was identified as being an indicator species for CD (padj=0.09). Differential abundance analysis according to phenotype as per Montreal classification revealed that, compared to patients with the B1 phenotype, patients with the B2 and/or B3 have a higher abundance of *Escherichia/Shigella uncl.* (ASVs 13, 31, 282 and 422), *Klebsiella uncl.* (ASVs 75 and 101) and *Enterobacter uncl.* (ASV 219) (Figure 1). Furthermore, patients with L3 involvement had higher abundances of *Klebsiella uncl.* (ASVs 75 and 101) and *Parasutturella uncl.* (ASVs 22, 53, 120, 199, 249 and 510), the latter being a *Proteobacteria*, compared to patients with L1 and/or L2 involvement. No significant association with "Age of Onset" was identified. In addition, network analyses revealed a strongly correlated group of *Enterobacteriaceae* ASVs (*Klebsiella*, *Escherichia/Shigella*, *Enterobacter*, *Citrobacter*) which appear to collectively associate to CD.
Conclusion: *Enterobacteriaceae* persist in the faecal microbiota in significantly higher levels than controls despite remission and furthermore are associated with the more severe phenotypes of stricturing and penetrating disease. Further studies might indicate whether microbiota assessment on diagnosis might predict CD subtypes and therefore influence therapeutic choices.

DOP26

The relationship between vedolizumab therapeutic drug monitoring, biomarkers of inflammation, and clinical outcomes in Inflammatory Bowel Disease in the real-world setting

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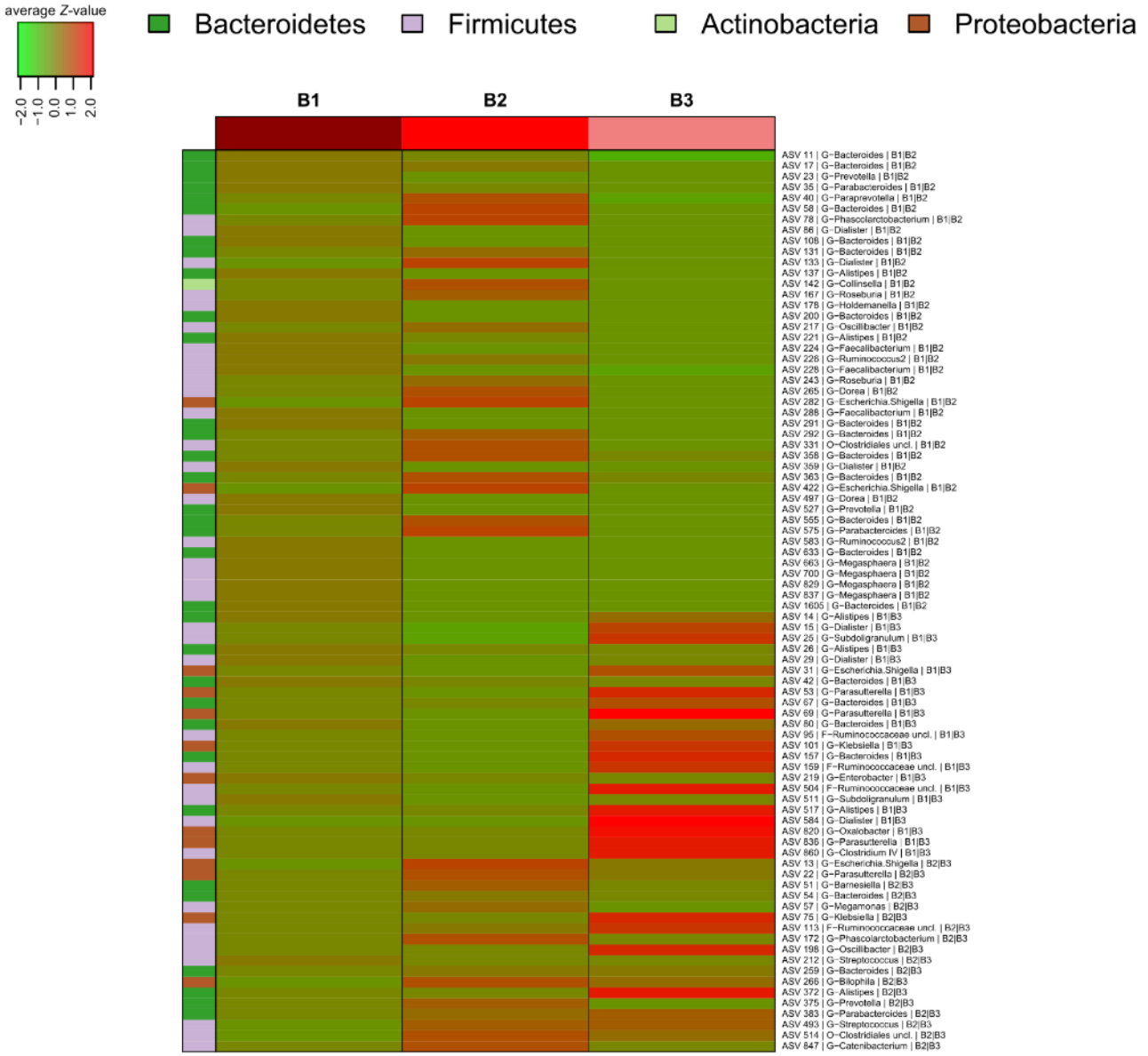
Background: Despite widespread use of therapeutic drug monitoring to guide anti-TNF biologic prescribing in IBD, its role for other biologic classes remains unclear. The present study aimed to assess the relationship between early vedolizumab trough concentrations (VTC) and real-world outcomes in inflammatory bowel disease (IBD).
Methods: Individuals with IBD enrolled in the Takeda Canada Patient Support Program were assessed at regular intervals from 2018–2020. VTC, albumin, faecal calprotectin (FC), C-reactive protein (CRP), and disease scores were collected from Crohn's disease (CD; Harvey-Bradshaw Index, HBI) and ulcerative colitis (UC; Partial Mayo scores, PMS) patients. The relationship between

Abstract DOP25 – Figure 1: Heatmap visualizing significant differentially abundant ASVs in CD patients with respect to behaviour subgroups



post-induction (Week 6) VTC, baseline albumin, and Week 30 remission as defined by CRP (<5mg/L), FC (<250µg/g), or disease scores (HBI <5; PMS <3) was assessed.

Results: Week 6 VTC levels were higher in IBD patients who achieved Week 30 CRP remission compared with those who did not (n=248, p=0.0004). The Week 6 VTC cut-off that best predicted CRP remission for all IBD patients was 39.65µg/mL (AUROC: 0.61, 95% CI: 0.54–0.69, p<0.001), with significance in both univariate (n=248; p<0.001) and multivariate (p=0.002) analyses. In disease-specific subgroups, optimal VTC cut-offs in CD and UC patients displayed this same relationship. UC patients with Week 6 VTC levels >39.65µg/mL had significantly longer treatment duration (n=115; HR: 0.29, 95% CI: 0.10–0.86); CD patients did not. Week 6 VTC levels were higher in IBD patients who achieved Week 30 FC remission compared with those who did not (n=170, p=0.003). The Week 6 VTC cut-off that best predicted Week 30 FC remission for all IBD patients was 41.9µg/mL (AUROC: 0.62, 95% CI: 0.54–0.7, p<0.0001), with significance in univariate (n=170; p=0.002) but not multivariate analyses. Baseline albumin levels in all IBD patients were independently associated with Week 30 FC remission by multivariate analyses (n=170, p=0.006). In disease-specific subgroups, optimal Week 6 VTC cut-offs and baseline albumin levels were associated with Week 30 FC remission in CD patients only. All IBD patients with Week 6 VTC levels >41.9µg/mL had longer treatment duration (n=170; HR: 0.31, 95% CI: 0.12–0.83). No relationships were found between Week 6 VTC and HBI or PMS disease scores.

Conclusion: Post-induction VTC predicts remission by CRP in individuals with CD and UC but not remission by FC or clinical disease indices. Baseline albumin is independently associated with FC remission. Further research is warranted to better understand these relationships, and to develop multivariable predictive tools that combine baseline biomarkers with early vedolizumab pharmacokinetics.