

Microbiome Beta-Glucuronidase Activity Does Not Limit Bioconversion or Pharmacokinetic Consistency of the Colon-Targeted PDE4 Prodrug PALI-2108 in Ulcerative Colitis

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Introduction and Aim

- Inflammatory bowel disease (IBD), including ulcerative colitis (UC), is an immune-mediated inflammatory disorder of the gastrointestinal (GI) tract characterized by alterations in the gut microbiome (dysbiosis). Despite available therapies, many patients fail to achieve sustained remission or experience treatment-limiting adverse effects.
- PALI-2108 is a novel, once-daily, colon-targeted oral phosphodiesterase-4 (PDE4) prodrug designed to be converted locally to its active metabolite, PALI-0008, by bacterial β -glucuronidase (GUS) activity within the colon, with the goal of maximizing local drug exposure while minimizing systemic exposure.
- Because UC-associated dysbiosis and inter-individual differences in microbial composition and GUS activity may influence prodrug activation, variability in bioconversion could contribute to differences in pharmacokinetic (PK) exposure and treatment response.
- We evaluated gut microbiome composition, GUS-associated microbial features, and PK variability in healthy/asymptomatic volunteers (HVs) and UC subjects enrolled in the Phase 1a/b clinical development program to elucidate factors influencing PALI-2108 activation and exposure.

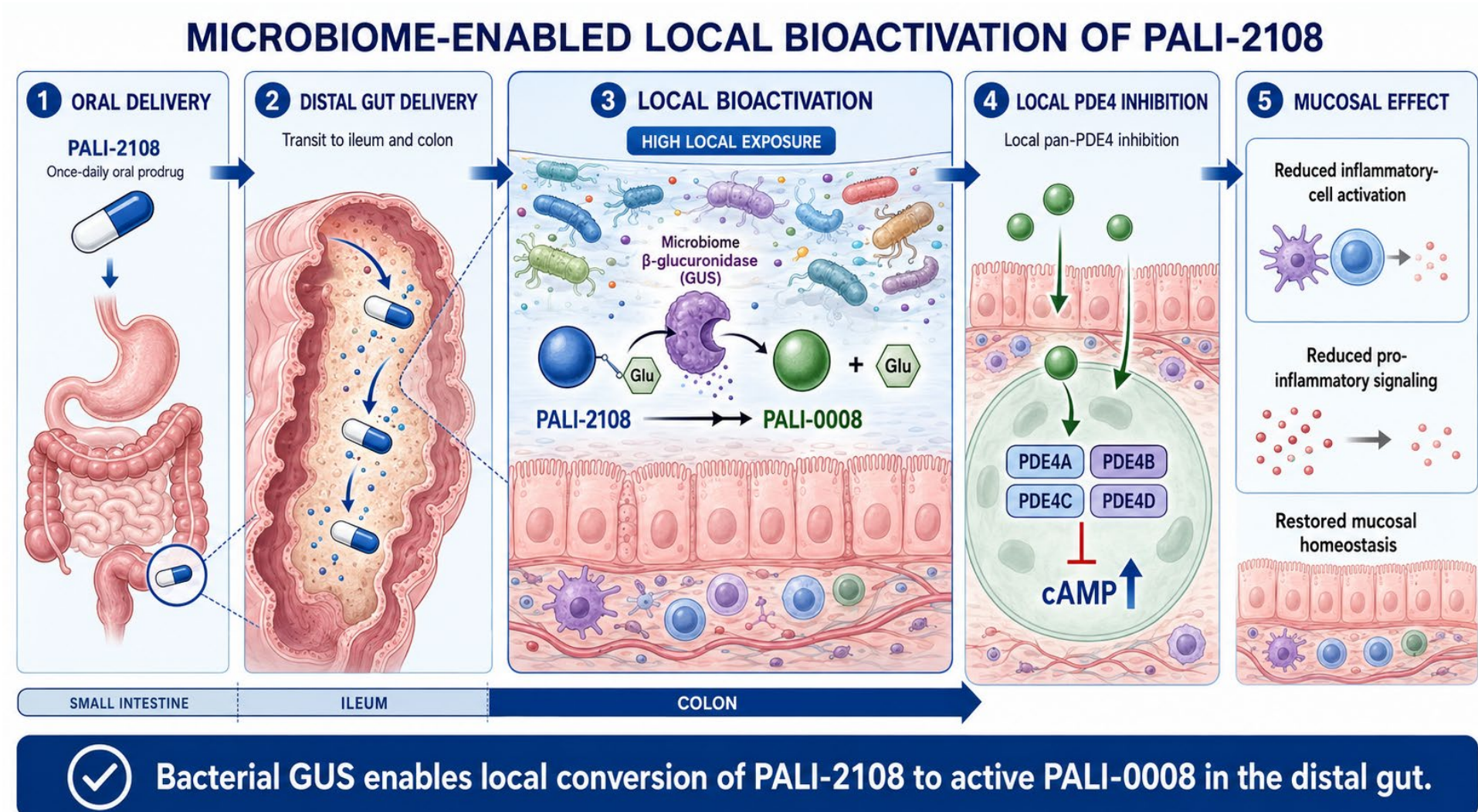


Figure 1: Localized bio-activation of PALI-2108 targets the ileum and colon, reducing the risk of GI and CNS adverse events versus non-specific and non-localized PDE4 inhibitors.

Methods

- Shotgun metagenomic sequencing of baseline stool samples from healthy volunteers (HVs) and patients with UC was used to characterize bacterial species and MetaCyc functional pathways.
- Microbiome diversity and differential functional analyses (GO, EC, and GUS-associated features) compared HV vs UC and longitudinal changes in UC following treatment.
- Cross-species/public metagenomic datasets (mouse, dog, healthy human, Crohn's disease (CD), and UC) were harmonized to assess conservation and disease-associated differences in GUS-related functions.
- PALI-0008 PK variability was assessed using Phase 1a/b normal HV and fibrostenotic Crohn's disease (FSCD) exposure data and compared with published apremilast and mufemilast population PK data to contextualize exposure consistency following microbiome-dependent prodrug activation.

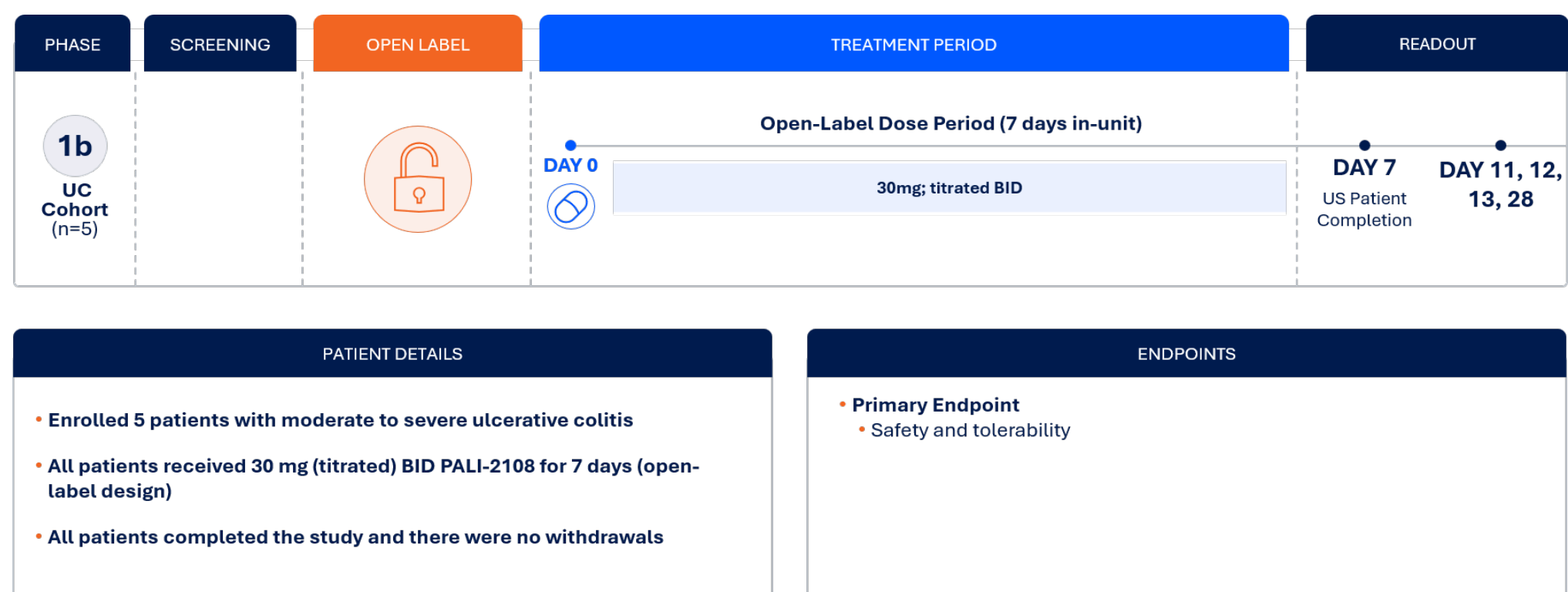


Figure 2: Phase 1b study of PALI-2108 in UC patients (NCT06663605).

β -glucuronidase Functional Capacity is Preserved Across Preclinical and Clinical Cohorts

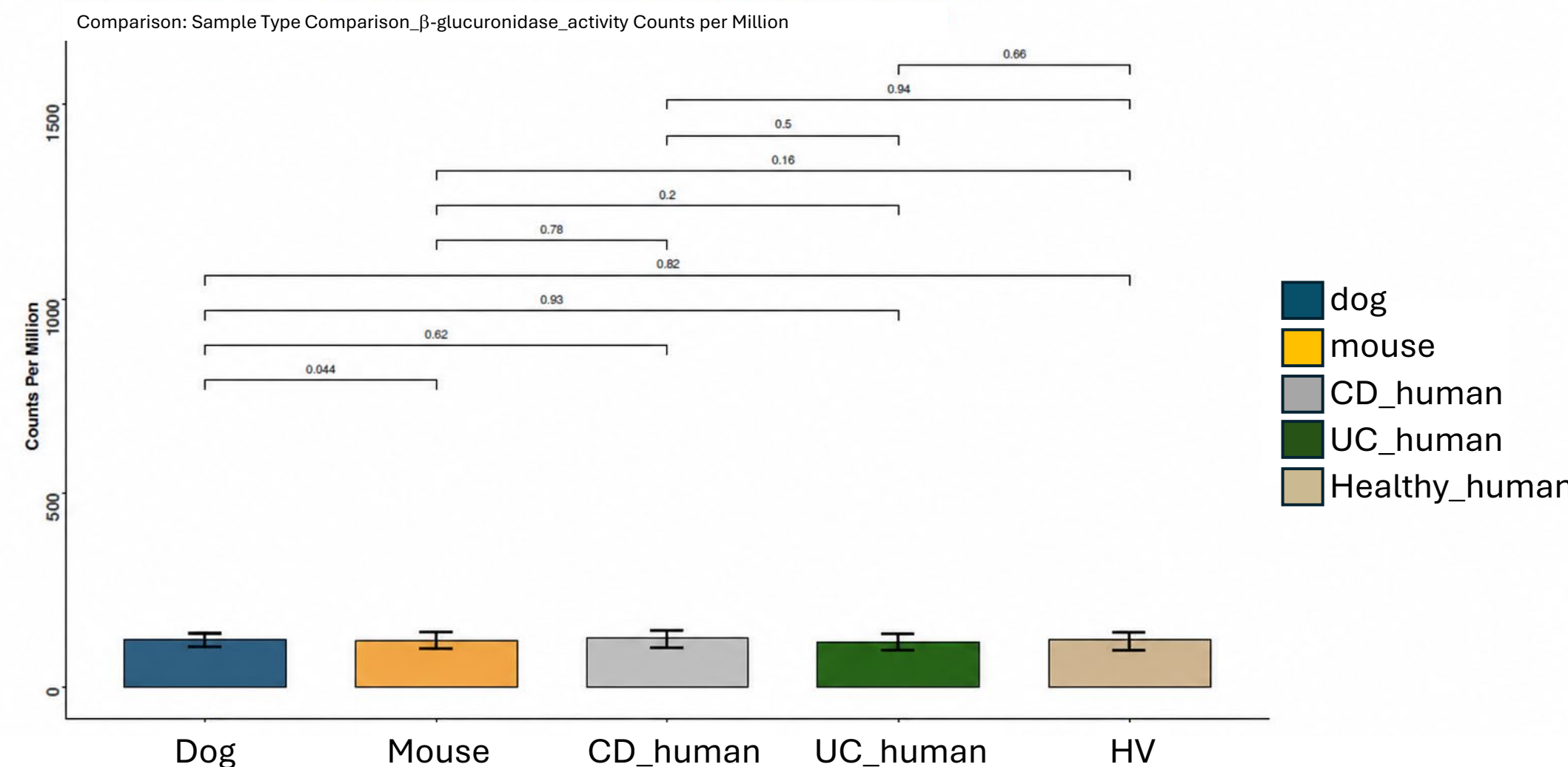


Figure 3: Cross-species analyses demonstrated consistent detection of EC and GO GUS-related features with no significant pairwise differences after batch correction. CD, Crohn's disease; EC, Enzyme Commission; GO, Gene Ontology; GUS, β -glucuronidase; HV, healthy volunteer; UC, ulcerative colitis

UC Subjects Exhibit Comparable β -glucuronidase Relative Abundance to Healthy Volunteers

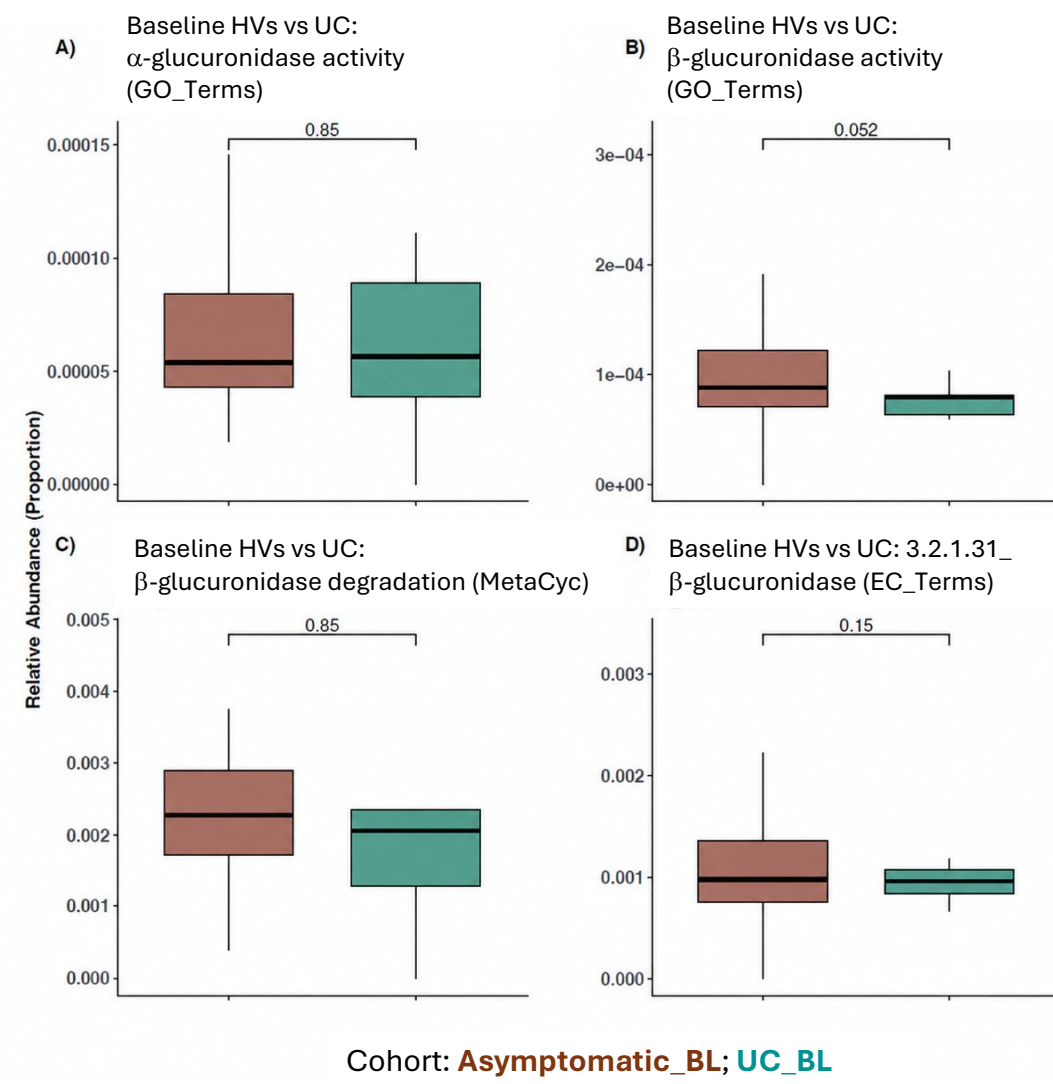


Figure 4: The relative abundance of functional features associated with GUS are shown for each cohort. The data was aligned to GO (A, B), MetaCyc®, and EC (C, D) functional databases to identify GUS genes. Wilcoxon rank-sum test were used to test for significant differences between cohorts. BL, baseline; EC, Enzyme Commission; GO, Gene Ontology; GUS, β -glucuronidase; HV, healthy volunteer

β -glucuronidase-Associated Functional Features are Preserved in UC Patients and Remain Stable Throughout Treatment

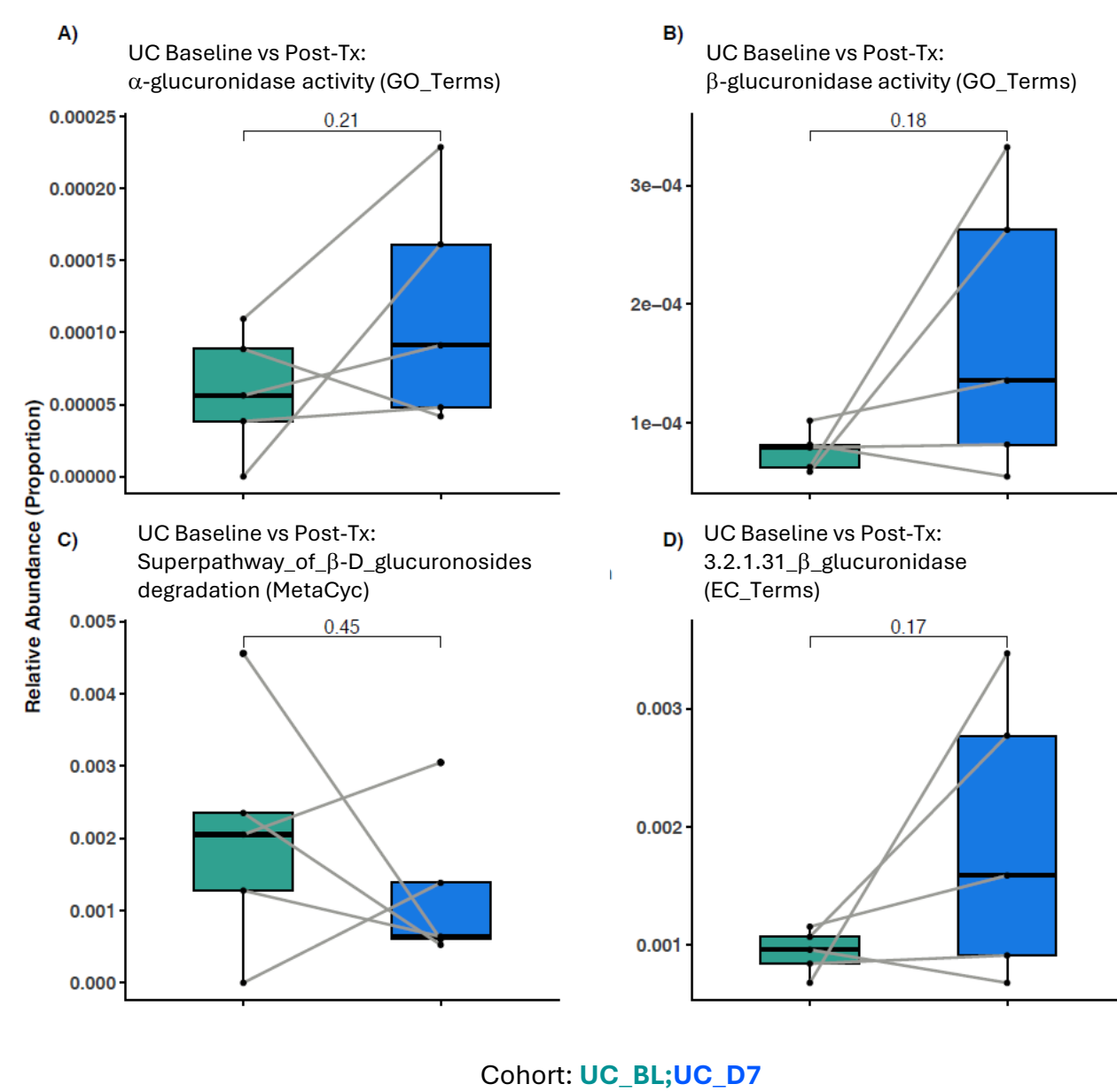


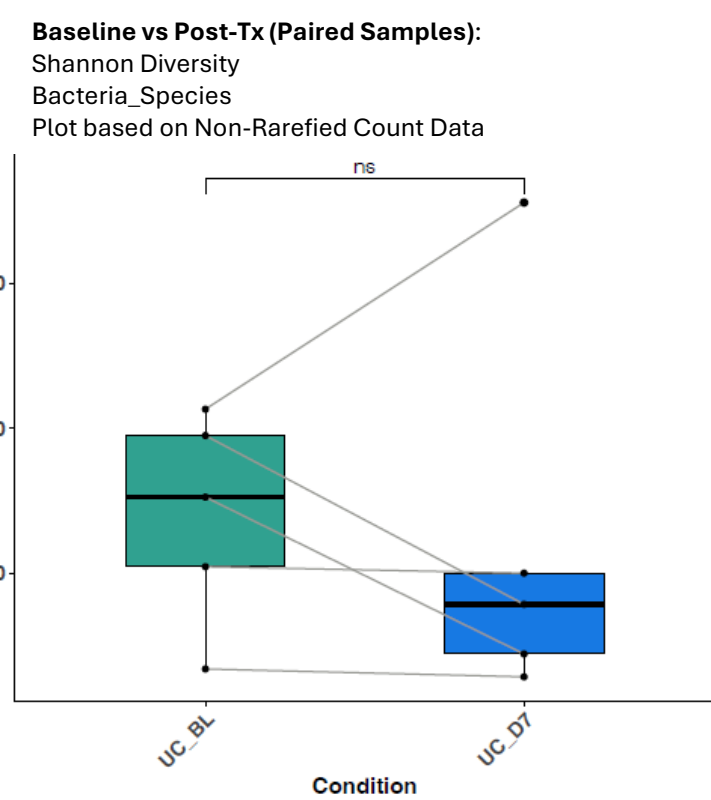
Figure 5: Changes in GUS-associated functional features following PALI-2108 treatment in UC. Relative abundance of GUS-associated GO terms (A, B), the MetaCyc β -D-glucuronosides degradation pathway (C), and EC β -glucuronidase annotation (D) at baseline and post-treatment. Lines indicate paired samples. EC, Enzyme Commission; GO, Gene Ontology; GUS, β -glucuronidase; Tx, treatment; UC, ulcerative colitis

Results

Treatment with PALI-2108 Does Not Change Alpha or Beta Diversity in UC Patients Over 1 Week

Alpha Diversity - Shannon Diversity Index

UC Samples Over Time - Bacteria Species



Beta Diversity - Bray-Curtis Beta Dissimilarity

UC Samples Over Time - Bacteria Species

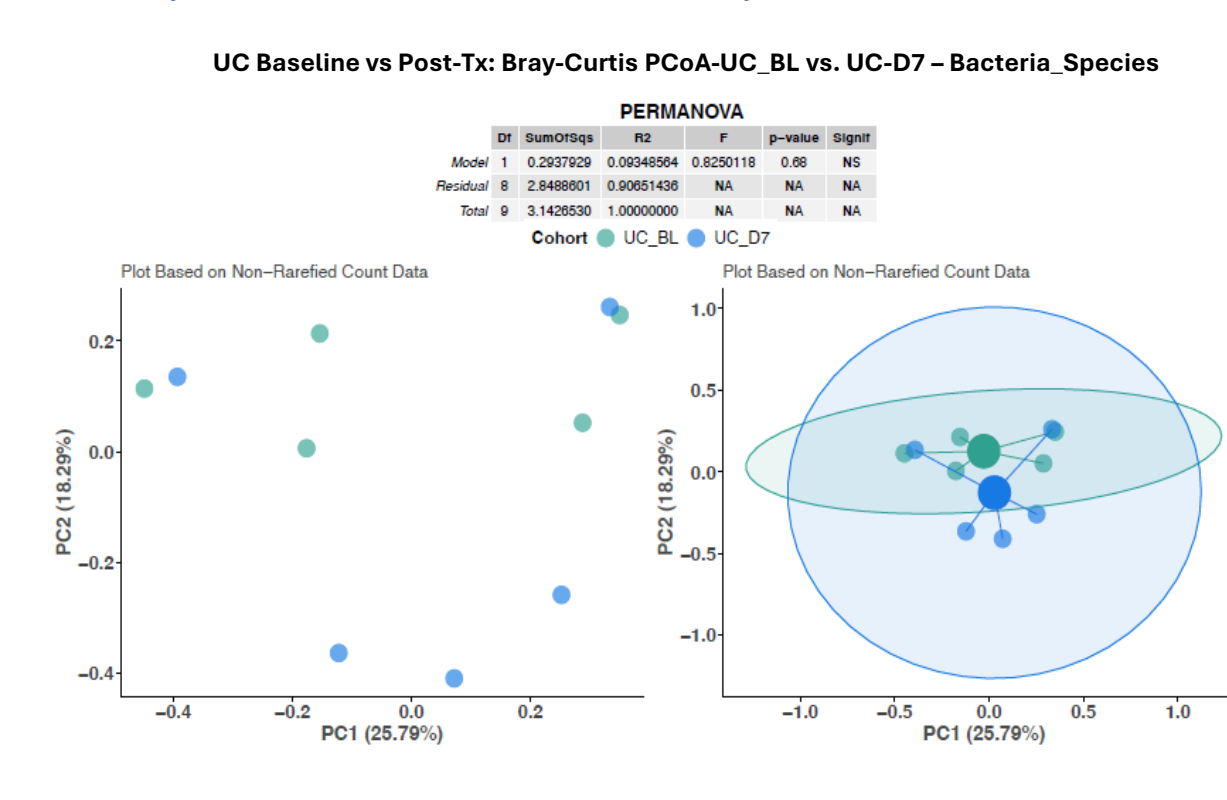


Figure 6: Shannon alpha diversity (richness and number of unique features) and Bray-Curtis beta diversity (feature presence/absence) were not significantly different over the course of the study. BL, baseline; D7, day 7; Tx, treatment; UC, ulcerative colitis

β -glucuronidase-Bioactivated PALI-0008 Demonstrates Lower C_{Trough} Variability Comparable to Conventional Oral PDE4 Inhibitors in NHV and FSCD Cohorts

(A) NHV+FSCD pooled, dose $\leq$ 30mg

(B) NHV+FSCD pooled, 30mg only

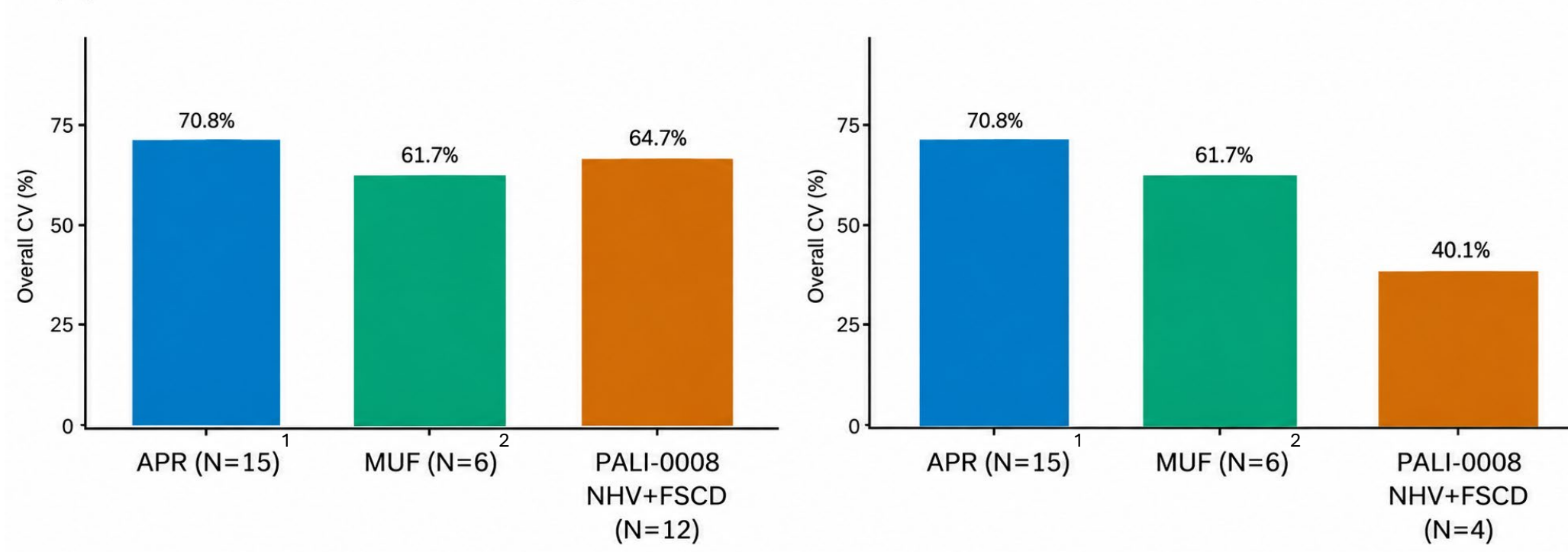


Figure 7: C_{Trough} CV%: Apremilast (APR) vs. Mufemilast (MUF) vs. PALI-2108 in fibrostenotic Crohn's disease (FSCD) cohort. APR 30mg BID Day 7 SS PsA (N=15), MUF 60mg BID Day 9 SS HV fasted (N=6) compared with (A) PALI-0008 (N=12) $\leq$ 30mg: NHV F1 15mg Day 10 (n=4), NHV F2 30mg Day 10 (n=3), FSCD E1 20mg Day 11 (n=2), FSCD E2 25mg Day 11 (n=2), FSCD E3 30mg Day 11 (N=1); (B) PALI-0008 (N=4) = 30mg: NHV F2 30mg Day 10 (N=3), FSCD E3 30mg Day 11 (N=1). BID, twice daily; FSCD, Fibrostenotic Crohn's disease; NHV, normal healthy volunteer; PsA, psoriatic arthritis; SS, steady state

1. FDA CDER. Apremilast Clinical Pharmacology Review, NDA 205437. 2014.
2. Li X, et al. Mufemilast first-in-human phase 1 study. Clin Pharmacol Drug Dev. 2026;15:e70005. doi:10.1002/cpdd.70005.

Summary

- PALI-2108 was effectively converted to active PALI-0008 in both healthy volunteers and patients with UC, supporting robust microbiome-mediated activation.
- Gut microbiome composition and GUS-associated features were similar between healthy volunteers and UC subjects, with no significant treatment-related changes in GUS activity.
- Cross-species analyses demonstrated conserved GUS-associated functions, supporting the translational relevance and stability of microbiome-mediated activation.
- PALI-0008 exposure was reproducible, with PK variability comparable to or lower than published apremilast data in HVs and FSCD cohorts, indicating that dysbiosis does not impair PALI-2108 bioconversion.

