MICROBIOME CHANGES ASSOCIATED WITH NITAZOXANIDE IN A RANDOMIZED, DOUBLE-BLIND STUDY FOR THE TREATMENT OF CHRONIC NOROVIRUS INFECTIONS IN SOLID ORGAN TRANSPLANT RECIPIENTS

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NOROVIRUS INFECTION IN IMMUNOCOMPROMISED PATIENTS

- **Diarrhea** is a commonly encountered problem in **Solid-organ transplant** (SOT) recipients, caused by a wide array of both infectious (bacterial, parasitic, and viral pathogens) and noninfectious etiologies
- **Gastroenteritis caused by human norovirus**, NoV, in SOT recipients accounts for significant morbidity and mortality
- There are **few therapeutic strategies for treatment of chronic norovirus**, with currently 2 clinical trials:
  - Nitazoxanide for Norovirus in Transplant Patients Study - completed
  - Adoptive T Lymphocyte Administration for Chronic Norovirus Treatment in Immunocompromised Hosts (ATLANTIC) - ongoing

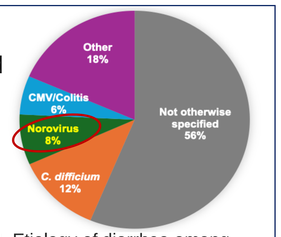


Fig 1. Etiology of diarrhea among hospitalized SOT recipients, (adapted from Ignacio A., et al. 2015)

- **Nitazoxanide (NTZ)**
  - **FDA-approved for diarrhea** caused by *Cryptosporidium* and *Giardia* by targeting the **anaerobic respiration** by inhibiting the pyruvate oxidoreductase (PFOR) enzyme
  - **Used off-label for several viruses** based on putative antiviral activity against influenza, rotavirus, norovirus, Ebola
  - In the past clinical studies to assess the antiviral activity of NTZ on NoV show conflicting results, ranging from improvement in clinical symptoms to no improvement at all

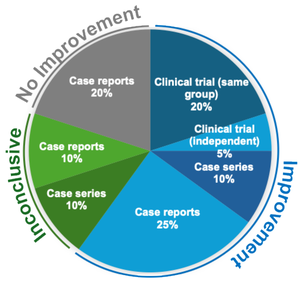


Fig 2. Summary of outcomes from 20 clinical studies using NTZ for treatment of chronic NoV gastroenteritis

- **Nitazoxanide for Norovirus in Transplant Patients Study**

Study design: Phase 2 multi-center, prospective, randomized, double-blind study

Aim: Assess the clinical and antiviral efficacy and safety of NTZ for the treatment of NoV in SOT recipients

Methodology: Subjects with a positive NoV test within 14 days of enrollment and active GI symptoms were randomly assigned (1:1) to NTZ 500 mg twice daily or placebo (P) for 56 consecutives doses and were followed for 6 months

Variable	Characteristics	Placebo (N=15)	NTZ (500 mg) (N=16)
Female		8	7
Ethnicity	Not Hispanic or Latino	12	13
	Hispanic or Latino	1	2
Race	Black or African American	2	4
	White	13	11
Duration of symptoms	Acute (<14 days)	4	3
	Chronic (>14 days)	12	12

Conclusion: NTZ did not shorten time to clinical resolution or viral shedding duration but may have resulted in transient symptom improvement (Boutin, Catherine-Audrey, et al., 2023)

AIM & HYPOTHESIS

Aim: Since NTZ treated patients experienced transient symptom resolution, we aimed determine if NTZ administration is correlated to any microbiome changes observed in the stool of the SOT patients compared those of the placebo

Hypothesis: We hypothesized that **NTZ maybe modulating the gut microbiome** in these patients, potentially contributing to transient symptom improvement, which is independent of its antiviral activity

STUDY SAMPLE & METHODS

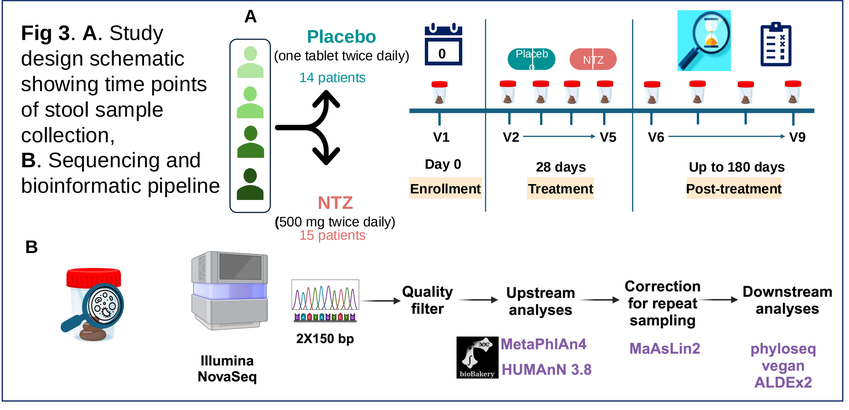


Fig 3. **A**. Study design schematic showing time points of stool sample collection, **B**. Sequencing and bioinformatic pipeline

NITAZOXANIDE TREATMENT RESULTS IN A CHANGE IN MICROBIOME IN IMMUNOCOMPROMISED PATIENTS WITH CHRONIC NOROVIRUS INFECTIONS

Samples at "Enrollment" phase have similar microbiome composition

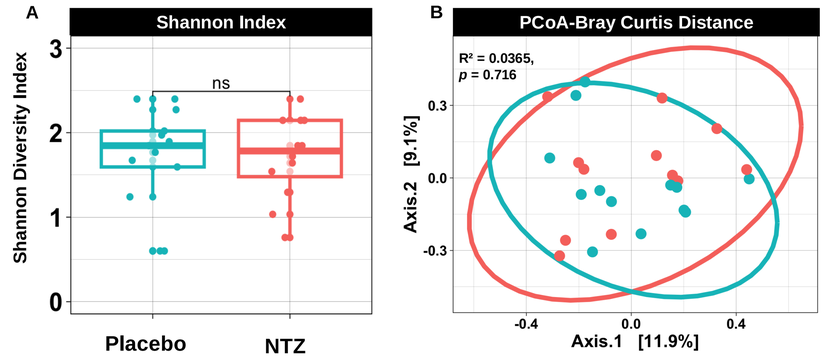


Fig 4. **A**. Alpha diversity and **B**. Beta-diversity plot showing no significant difference in overall microbiome composition between **Placebo** and **NTZ** group at enrollment

Significant difference in microbiome during the "Treatment" phase between placebo and NTZ group

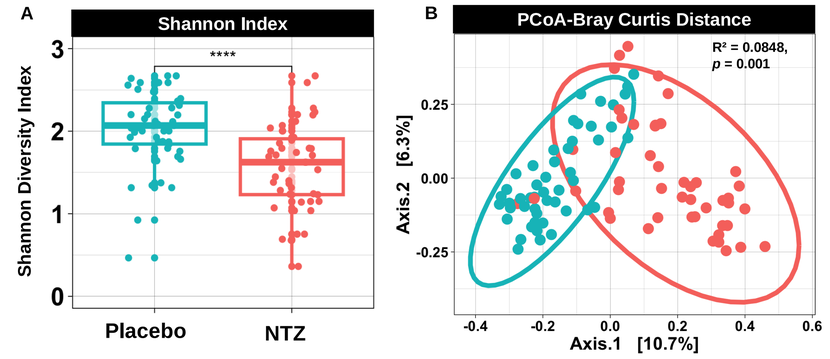


Fig 5. **A**. Alpha diversity and **B**. Beta-diversity plot showing significant difference in overall microbiome composition between **Placebo** and **NTZ** group at the treatment phase

Differences in microbiome resolves during the "Post-treatment" phase between placebo and NTZ group

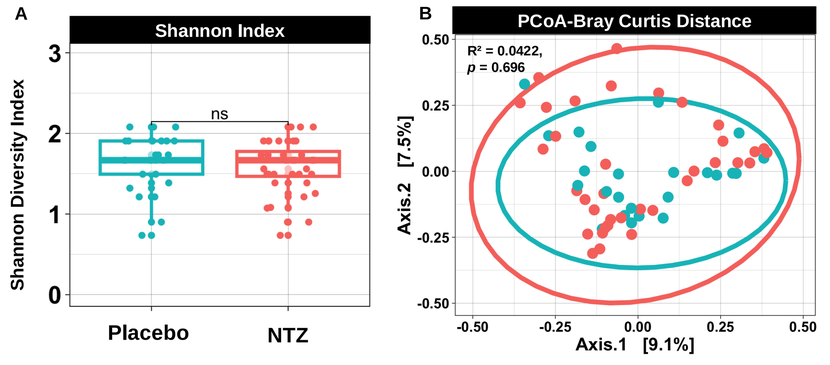


Fig 6. **A**. Alpha diversity and **B**. Beta-diversity plot showing no significant difference difference in overall microbiome composition between **Placebo** and **NTZ** group at the post-treatment phase

NTZ treatment is associated with changes in taxonomic distribution during "Treatment" phase

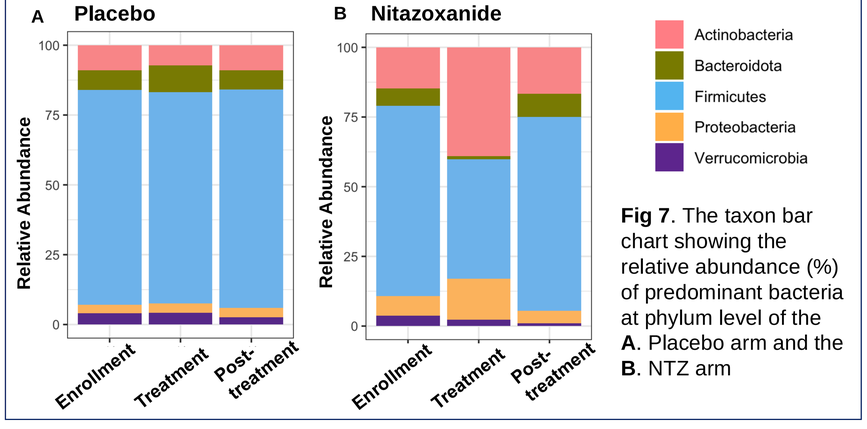


Fig 7. The taxon bar chart showing the relative abundance (%) of predominant bacteria at phylum level of the **A**. Placebo arm and the **B**. NTZ arm

NTZ treatment induces an increase in *Blautia wexlerae* and decease in *Bifidobacterium dentium*

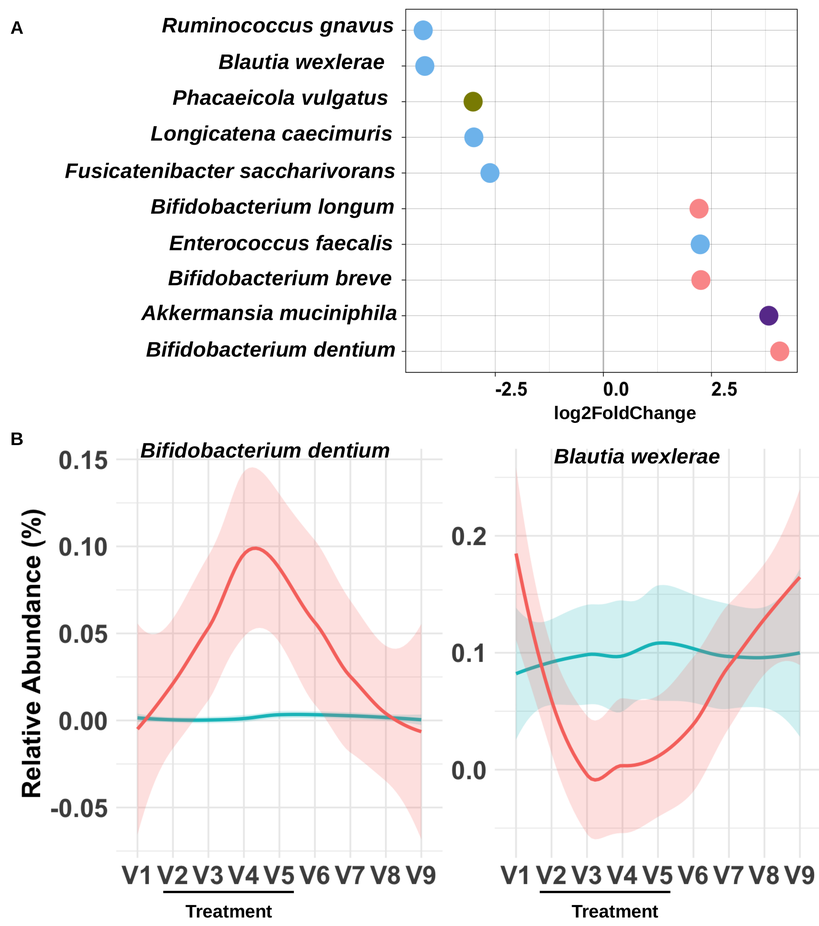
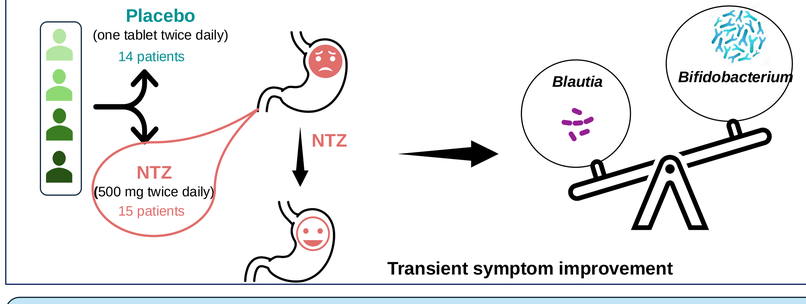


Fig 8. **A**. Differential abundance analysis of species changes between "Treatment" and "Post-treatment" phase of the NTZ arm showing log2-fold change analyzed by DESeq2 differential abundance analysis **B**. Relative abundance distribution of selected species between **Placebo** and **NTZ** group across all the 3 phases

SUMMARY & FUTURE DIRECTION

- Baseline microbiome composition was similar between the placebo and NTZ treatment groups
- NTZ administration led to significant changes in taxonomic diversity, affecting both alpha and beta diversity
- Differential abundance analysis showed
  - **increase in *Bifidobacterium dentium***, which by itself and also the factors generated by several other species of this genera are known to improve diarrheal symptoms
  - **decrease in *Blautia wexlerae*** was observed since it is an anaerobic bacteria that can metabolize pyruvate using the PFOR enzyme which is the target for NTZ
- Pathway analyses are currently underway to explore the functional implications of these microbial shifts



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