Investigating the Gram-Positive Selective Spectrum of Ibezapolstat, a First-in-Class DNA Polymerase IIIC (Pol IIIC) Inhibitor

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REVISED ABSTRACT

Background: Ibezapolstat (IBZ) is a non-absorbable antimicrobial currently in phase 2 clinical trials for the treatment of *Clostridioides difficile* infection (CDI). *In vitro* and human studies have shown potent activity of IBZ against *C. difficile* but selective activity against other beneficial Gram-positive gut microbiota shown to reduce the risk of recurrent CDI. As the target DNA Pol IIIC enzyme is present in most Gram-positive species, the reasons for this selectivity are unclear. The purpose of this study was to assess the selectivity of IBZ against Gram-positive gut microbiota.

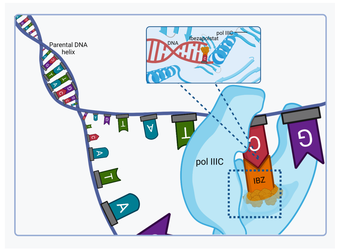
Methods: Using stool samples and microbiome data from the phase 1 and 2 studies, changes in proportional abundance and absolute quantities of gut Firmicutes were analyzed over time in healthy volunteers or patients with CDI given IBZ. Using isolated gut microbiota species, MIC determinations against a variety of isolated Gram-positive gut species were assessed by broth microdilution and whole genome sequencing.

Results: Baseline gut Firmicute microbiota from healthy volunteers were primarily Lachnospiraceae or Ruminococcacaea or which Lachnospiraceae were preserved throughout the IBZ dosing period. Lachnospiraceae were also preserved and had increased abundance in phase 2a CDI study. Using qPCR, relevant groups of Clostridiales (C. coccoidies and C. leptum groups) were preserved during the phase I and 2 studies. Individual Firmicute species isolated from phase 2a samples demonstrated heterogeneous susceptibility to IBZ

Conclusion: Microbiome Firmicute changes with IBZ were dependent on underlying composition of the baseline microbiome but consistently demonstrated increased preservation or increased abundance of Lachnospiraceae and Clostridiales after starting therapy. IBZ microbiome data coupled with *in vitro* MIC determinations demonstrated persistence or regrowth of healthy microbiota associated with beneficial physiologic effects.

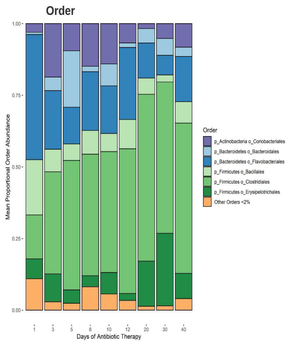
BACKGROUND

Ibezapolstat (IBZ): small-molecule inhibitor of DNA pol IIIC enzyme based upon competitive inhibition of dGTP (guanosine analog)



In Phase2a clinical trial, increased regrowth of certain Firmicutes was observed.

Why these beneficial microbes regrew is unknown given the MOA of IBZ



OBJECTIVE

To Investigate the selectivity of ibezapolstat against beneficial gut Firmicutes

1. Using data from the Phase 1 and Phase 2a clinical trials, to compare metagenomic differences between the studies.
2. Assess IBZ MIC from Firmicutes isolated from the stool of patients in the phase 2a study

METHODS

Study design

- **Phase 1 study:** Data from six healthy volunteers given IBZ 450 mg twice daily for 10 days
- **Phase 2a study:** Data from 10 subjects with CDI given IBZ 450 mg twice daily for 10 days

Laboratory Investigations

Stool metagenomics (Fig 1, top row)

- Shotgun metagenomic sequencing and 16S-rRNA
- Proportional changes of Firmicute Family assessed over time

qPCR (Fig 1, second row)

- Targeted primers to Clostridium cluster XIVa (*C. coccoides*) and Clostridium cluster IV (*C. leptum*)
- Quantitation of relevant Firmicute clusters assessed over time

Firmicute Stool isolation (Fig 2-2)

- Phase 2a samples
- Firmicute selective media, species confirmation by 16S rRNA Sanger sequencing

MIC determinations (Fig 2-1)

- Broth microdilution to determine IBZ MICs to Firmicutes

Whole genome sequencing (Fig 2-3)

- Illumina sequencing with SNP calling vs. reference strains

Relevant References

- McPherson et al. Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-recurrence Effects in *Clostridioides difficile* Infection. Antimicrob Agents Chemother 2022. PMID: 35862742
- Garey et al. Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with *Clostridioides difficile* Infection: A Phase 2a Multicenter Clinical Trial. Clin Infect Dis. 2022 PMID: 35134880
- Garey et al. Randomized, Double blind, Placebo controlled, Single and Multiple Ascending Dose Phase 1 Study to Determine the Safety, Pharmacokinetics, Food, and Fecal Microbiome Effects of Ibezapolstat Administered Orally to Healthy Subjects. J Antimicrob Chemother 2020 PMID: 32892222
- Xu et al. Discovery and development of DNA polymerase IIIC inhibitors to treat Gram-positive infections. Bioorg Med Chem 2019 PMID: 31221610

RESULTS

Figure 1. Metagenomic and qPCR Data from the Phase I and Phase 2a clinical trials

Lachnospiraceae Family proportion preserved in Phase 1 and Phase 2a studies

Clostridiales Family abundance preserved in Phase 1 and Phase 2a studies

PHASE 1 HEALTHY VOLUNTEER STUDY

A. Metagenomics. Proportion of isolated Firmicutes per day during study

	Healthy volunteers					
FAMILY	Baseline	3	5	8	10	12
Erysipelotrichaceae	2%	4%	7%	7%	18%	25%
Eubacteriales Family XIII						
Lachnospiraceae	26%	23%	21%	16%	24%	47%
Lactobacillaceae	.	.	.	12%	5%	6%
Peptostreptococcaceae	.	.	.	.	.	.
Ruminococcaceae	68%	37%	20%	7%	3%	4%
Streptococcaceae	.	27%	43%	49%	41%	14%
Veillonellaceae	.	3%	.	.	.	.
Other	4%	6%	9%	9%	9%	5%

Most commonly isolated Genus (species) during study:Streptococcaceae (Lactococcus lactis, Streptococcus thermophilus); Lachnospiraceae (Blautia, Roseburia); Ruminococcaceae (Faecalibacterium, Ruminococcus)

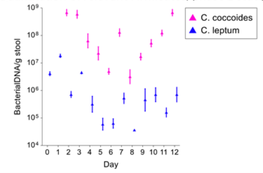
PHASE 2A CDI STUDY

B. Metagenomics. Proportion of isolated Firmicutes per day during study

	CDI patients					
FAMILY	Baseline	3	5	8	10	12
Erysipelotrichaceae	18%	24%	10%	9%	17%	6%
Eubacteriales Family XIII	.	2%	1%	3%	5%	4%
Lachnospiraceae	30%	37%	38%	33%	35%	44%
Lactobacillaceae	6%	.	.	.	.	.
Peptostreptococcaceae	2%	1%	.	5%	4%	6%
Ruminococcaceae	7%	25%	38%	30%	26%	24%
Streptococcaceae	13%	3%	1%	4%	5%	2%
Veillonellaceae	19%	.	.	5%	.	.
Other	5%	9%	10%	9%	8%	10%

Most commonly isolated Genus (species) during study:Streptococcaceae (Streptococcus salivarus, Streptococcus thermophilus); Lachnospiraceae (Blautia, Roseburia); Ruminococcaceae (Faecalibacterium, Ruminococcus)

C. qPCR. Quantitation of relevant Firmicutes, phase 1 study



D. qPCR. Quantitation of relevant Firmicutes, phase 2a study

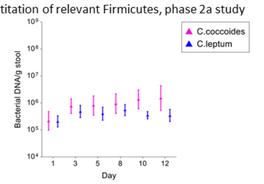


Figure 2. MIC Determinations and Whole Genome Sequencing of Resistant vs. Susceptible Strains

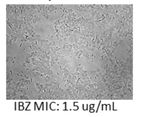
Beneficial Firmicutes have heterogeneous IBZ susceptibility

1. IBZ MIC Determinations of Firmicutes from the Phase 2a Study

Species	N	MIC50	Min	Max
Blautia sp.	1	12.5		
Clostridium butyricum	5	12.5	1.5	100
Clostridium subterminale	1	1.0		
Clostridium tertium	2	12.5	3.125	12.5
Coprococcus sp	1	25		
Dorea longicatena	1	3.125		
Enterococcus avium	2	12.5	12.5	12.5
Enterococcus durans	1	100	100	100
Enterococcus faecalis	5	12.5	12.5	50
Enterococcus mundtii	1	12.5		
Enterococcus pseudoavium	1	100		
Erysipelatoclostridium ramosum	5	6.25	3.125	100
Flavonifractor plautii	1	25		
Lachnoclostridium pacaense	1	25		
Longibaculum sp.	1	50		
Melissococcus plutonius	1	6.25		
Paeniclostridium sordellii	4	1.5	0.75	12.5
Clostridioides difficile	6	0.5	0.25	1

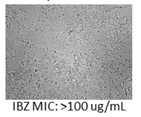
2. Isolation of same species with differing IBZ susceptibly

C. butyricum 1008



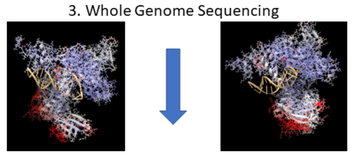
IBZ MIC: 1.5 ug/mL

C. butyricum 1007



IBZ MIC: >100 ug/mL

3. Whole Genome Sequencing



Sample	nucleotide position	nucleotide change	protein	protein change	COMMENTS
APT_1008	163003	A>C	WP_07198 2014.1	Tyr240Asp	OM_YfiO superfamily
APT_1007	904344	T>G	WP_07198 2178.1	Tyr38Asp	N-acetylmuramoyl-L-alanine amidase family protein
APT_1007	2512780	A>T	WP_07198 2697.1	Asp146Glu	serine protease

3 SNP difference resistant vs. susceptible strains (work in progress)

DISCUSSION AND CONCLUSIONS

- Beneficial microbiome effects of IBZ are most likely the results of preservation of Lachnospiraceae or Clostridiales species
- Targeted IBZ drug development towards *C. difficile* likely lead to heterogeneous activity against these beneficial commensals
- Future work will target MOA studies to elucidate targeted susceptibility towards *C. difficile*.

FUNDING

- Acurx Pharmaceuticals