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A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects

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Background: *Clostridioides difficile* infection is the most common cause of healthcare-associated infections in the USA, with limited treatment options. Ibezapolstat is a novel DNA polymerase IIIC inhibitor with *in vitro* activity against *C. difficile*.

Objectives and methods: Randomized, double-blind, placebo-controlled study to assess the safety, tolerability and pharmacokinetics of ibezapolstat in healthy volunteers. Microbiome changes associated with ibezapolstat were compared with vancomycin over a 10 day course using shotgun metagenomics.

Results: A total of 62 subjects aged 31 ± 7 years (45% female; average BMI: 25 ± 3 kg/m^2) were randomized. Ibezapolstat was well tolerated with a safety signal similar to placebo. Ibezapolstat had minimal systemic absorption with the majority of plasma concentrations less than 1 μg/mL. In the multiday, ascending dose study, ibezapolstat concentrations of 2000 μg/g of stool were observed by Day 2 and for the remainder of the dosing time period. In the multiday, multiple-dose arm, baseline microbiota was comparable between subjects that received ibezapolstat compared with vancomycin. At Day 10 of dosing, differential abundance analysis and β-diversity demonstrated a distinct difference between the microbiome in subjects given vancomycin compared with either dose of ibezapolstat ($P = 0.006$). α-Diversity changes were characterized as an increase in the Actinobacteria phylum in subjects that received ibezapolstat and an increase in Proteobacteria in subjects given vancomycin.

Conclusions: Ibezapolstat was shown to be safe and well tolerated, with minimal systemic exposure, high stool concentrations and a distinct microbiome profile compared with oral vancomycin. These results support further clinical development of ibezapolstat for patients with *C. difficile* infection.

Introduction

Clostridioides difficile infection (CDI) is the most common cause of healthcare-associated infections in the USA.[1] CDI is characterized by disruption of the host microbiome usually caused by prior use of high-risk antibiotics.[2] The dysbiosis allows germination of spores in the small intestine and production of two active toxins in the colon that cause disease.[3] Antimicrobial therapy is the hallmark of treatment, although there are limited treatment options. Historically, metronidazole has been widely used for the treatment of CDI but it is no longer recommended due to unacceptably high failure rates compared with vancomycin, higher mortality and cumulative toxicity.[4,5] Vancomycin is recommended by the guidelines[4] but is associated with a high rate of CDI recurrence and has recently been shown to have increased resistance due to profound disruption of the host microbiota.[6,7] Fidaxomicin has a lower recurrence rate but resistance has been shown via mutations in the *rpoB* gene.[8] Thus, new therapies with distinct mechanisms of actions directed against *C. difficile* are urgently needed.

Ibezapolstat (ACX362E) is a DNA polymerase IIIC inhibitor with potent activity against *C. difficile*.[9] This new class has a mechanism of action distinct from other currently available antimicrobials. The DNA polymerase IIIC enzyme is essential for replication of low-G + C content (fewer G and C DNA bases than A and T bases) Gram-positive bacteria and thus should be selective for Firmicutes such as *C. difficile* yet inactive against other host microbiota such as Actinobacteria or Bacteroidetes. Ibezapolstat was minimally absorbed in the hamster model, leading to high colonic and low systemic concentrations, and was also shown to be effective for CDI.[10] Preclinical safety testing demonstrated a favourable safety profile. These preclinical findings justified progression to a Phase 1 clinical trial. The objective of this study was to assess the safety, tolerability and pharmacokinetics associated with single and multiple oral doses of ibezapolstat. Microbiome changes associated with ascending doses of ibezapolstat were compared with standard-dose vancomycin following repeat oral administration over a 10 day course.

Materials and methods

Ethics

This study was performed in compliance with International Council for Harmonisation Good Clinical Practice, including the archiving of essential documents, as well as the ethical principles of the Declaration of Helsinki. This study was granted ethics approval by the Midlands Institutional Review Board (IRB# 220170383). All subjects signed an informed consent at screening. Subjects were given unique study identifiers to assure that their privacy was safeguarded.

Study drugs

Ibezapolstat 150 mg capsules (lot 1575AA01) and matching placebo (lot 7575AB01) were manufactured according to Good Manufacturing Practices and supplied by Piramal Pharma Solution, Ahmedabad, India. Individual capsules of ibezapolstat or placebo were packaged into high-density polyethylene bottles with integral seals and caps. Individual doses were taken from these bottles according to randomized allocation. Commercially available vancomycin HCL Pulvules (lot 503674) were purchased from the manufacturer (Eli Lilly and Company, Indianapolis, IN, USA).

Design and objective

The study was conducted in a Clinical Research Unit (CRU) by Altasciences Clinical Research, Overland Park, KS, USA. Clinical laboratory evaluations were performed by Quest Diagnostics, Lenexa, KS, USA. Bioanalysis of plasma and stool specimens was performed by Altasciences Inc., Montreal, Canada. Pharmacokinetics of ibezapolstat (plasma and stool) were performed by Learn and Confirm, St-Laurent, Quebec, Canada. The analytical range of ibezapolstat was 20.0–4000.0 ng/mL in plasma and 2.50–500.00 μg/g in stool. Pharmacokinetics of vancomycin (stool) and microbiome analysis were determined at the University of Houston College, Houston, TX, USA.

This was a three-part, randomized, placebo-controlled study. Subjects and investigators were blinded to ibezapolstat or placebo. Vancomycin was used as a comparator for microbiome analysis and was given in open-label fashion in Part 3 of the study. Part 1 was an ascending dose study, Part 2 was a food effect study, and Part 3 was a multiday, ascending dose study (Table 1). Sample size was typical for Phase 1 antibiotic studies to evaluate safety, pharmacokinetics and microbiome changes in healthy volunteers.

The primary objective of the study was to assess the safety and tolerability of ibezapolstat in both ascending single- and multiple-dose administration to healthy subjects. Safety and tolerability were assessed by adverse event (AE) monitoring, physical examinations, electrocardiograms and clinical laboratory evaluations. Safety evaluations were performed during dosing and for 3 days after the last dose. Subjects in the multiday, ascending dose study were also seen at a Day 32 follow-up visit. The secondary objectives were to assess pharmacokinetic changes associated with food, to determine systemic and faecal pharmacokinetics of ibezapolstat in both single- and multiple-dose administration, and to determine the faecal microbiome effects of ibezapolstat compared with those of oral vancomycin.

Parts 1 and 3

In Part 1 a single, ascending dose of ibezapolstat 150, 300, 600 or 900 mg ($n = 6$ per group) or placebo ($n = 2$ per group) was given. Thirty-two subjects were housed in the CRU from Day −1 (the day prior to the first dose administration) until 96 h after the dose was given (Day 4). The dose was given after an overnight fast. A minimum of 7 days occurred between each dose escalation to review safety and tolerability data. In Part 2 a single dose of ibezapolstat 300 mg was given in a fasted state or with food. Eight subjects were housed in the CRU using the same protocol as Part 1. After a 7–14 day washout period, subjects fasted for at least 10 h overnight, followed by a high-fat high-calorie breakfast in the morning of Day 1. Subjects were asked to consume the entire meal within 30 min. Ibezapolstat was administered 30 min after the start of the breakfast. Subjects were then asked to fast (no food or liquid, except water starting 1 h after dose administration) for an additional 4 h. Part 3 was a multiple-day, ascending dose study of ibezapolstat 300 and 450 mg given twice daily ($n = 6$ per group), placebo ($n = 2$ per group) or vancomycin 125 mg given four times daily ($n = 6$). The morning dose of study drug was given after an overnight fast. The afternoon dose

Table 1. Summary of dosing and group design for the randomized, placebo-controlled study

	Part 1	Part 2	Part 3
Design	single, ascending dose	food effect crossover	multiple, ascending dose (MAD)
Treatment days	1 dose	1 dose	10 days (20 doses)
Dose cohort	150, 300, 600, 900 mg	300 mg	300, 450 mg
N	6 per cohort	8	6 per cohort
Comparator (*n*)	placebo (2)	none	vancomycin (6) placebo (2)
Purpose	safety PK (systemic and stool)	safety PK (systemic and stool)	safety PK (systemic and stool) microbiome

PK, pharmacokinetic.

was given at least 1 h before and 2 h after eating. The additional two doses of vancomycin were given 6 h after the morning and evening doses.

Study subjects

The trial population was healthy male or female subjects aged 18–45 years (inclusive) with a BMI between 18.5 and 29.9 kg/m^2 (inclusive), who provided informed consent, and were healthy as determined by the principal investigator based on a medical evaluation including medical history, physical examination, safety laboratory tests and ECG monitoring. Subjects were not included in the study if they had clinically significant medical conditions, a history of malignancy except low-grade skin cancer, positive serology results for HIV, hepatitis B surface antigen or HCV antibodies, any gastrointestinal disease or disorder of gut motility that could interfere with the study objectives, use of antibiotics in the last 28 days or a history of known CDI within the past year.

Randomization and blinding

Treatment randomization code was produced by the statistician at the CRU independent from study investigators. On the day prior to dosing when subjects arrived at the CRU location, subjects were randomly allocated to a treatment in the cohort using a computer-generated pseudo-random permutation procedure. The randomization schedule was kept secure from blinded study staff until all study procedures were completed and the study database was locked. The randomization list was kept secure in the CRU pharmacy in case it was required to break the code. However, all investigators and subjects remained blinded until the database was closed.

Safety and tolerability assessments

The tolerability and safety of ibezapolstat were evaluated based on AE reports, vital signs, ECGs, safety laboratory values and results of physical examination. Study subjects were monitored carefully throughout each dosing period for adverse experiences. The relationship of AEs to the study treatment was assessed by the investigator based on temporal relationship to study treatment administration, subject's relevant medical history and presence of pre-existing conditions. Nature, time of onset, duration, severity and possible relationship to study medication were documented.

Vital signs and ECG

Oral temperature, heart rate and blood pressure were assessed at baseline at specified times during the study, and at other times if judged to be clinically appropriate. Blood pressure was assessed while the subject was in the supine position after an appropriate period of rest (3–5 min).

Triplicate 12-lead ECGs were obtained at baseline and then at specified times during the study, and at other times if judged to be clinically appropriate. ECGs were obtained after the subject had been in a supine position for at least 10 min. Triplicate ECGs were obtained within 10 min of each other.

Clinical laboratory evaluations

Blood and urine were collected at baseline and at specified time periods during the study to assess haematology, clinical chemistry, coagulation, urinalysis, viral serology, pregnancy and drug and alcohol screen.

Blood and faecal pharmacokinetic sampling

Pharmacokinetic blood samples for single-dose studies (Part 1 and Part 2) were collected as follows: Day 1 [pre-dose (within 1 h of dosing) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 16 h post-dose]. Additional samples were taken 24, 36 and 48 h post-dose.

Full pharmacokinetic blood samples for the multiday, ascending dose study (Part 3) were collected on Day 1 and Day 10 as follows: pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 11, 12 and 16 h relative to the Day 1 and Day 10 doses. Pre-dose blood samples were also drawn on Days 2, 7, 8, 9, 10, 11, 12 and 13. Concentrations of ibezapolstat in plasma samples were determined by LC–MS/MS.

Faecal samples for microbial analysis were collected pre-dose and daily, if available. As it was not guaranteed that a faecal sample would be produced on Day 1 pre-dose, subjects were requested to bring a faecal sample to the study centre at entry. Faecal samples were homogenized and concentrations of ibezapolstat or vancomycin were assessed by LC–MS/MS.

Gut microbiota analysis

Stool DNA extraction

Stool DNA was extracted by using a DNeasy PowerSoil Pro Kit (Qiagen, catalogue number 12888-100) in a QiaCube automated DNA extraction system according to instructions. Briefly, 250 mg of stool was transferred into a PowerBead Pro Tube provided with the kit and 200 μg of RNaseA and 800 μL of CD1 solution was added. Tubes were vortexed briefly, transferred into an adapter and then vortexed at maximum speed for 10 min. Tubes were centrifuged at 15000 **g** for 1 min and about 500–600 μL of supernatant was used for DNA extraction according to instructions. DNA was eluted in 70 μL of elution solution C6 and stored at −80°C until use.

Quantitative PCR analysis

Quantity and quality of extracted DNA were assayed with a Qubit 4 Fluorometer (Invitrogen). Sample DNA was diluted with PCR-grade water to 5 ng/μL. The DNA levels of bacterial groups were assessed using specific PCR primers/conditions.[11–14] Using the 7300 Real Time PCR System (Applied Biosystems), quantitative PCR (qPCR) was performed on each sample in triplicate in a final volume of 20 μL containing 25 ng DNA template, primers at 0.5 μM and QuantiTect SYBR Green Mixes (Qiagen). For Eubacteria an FAM-tagged probe at 0.25 μM and TaqPath ProAmp Master Mixes (Qiagen) were used. Threshold cycle values were converted into copies per ng of DNA using a standard curve. Standards were prepared by performing PCR using species-specific primers on appropriate bacterial strains or DNA from normal stool. The PCR products were cloned using the Invitrogen TOPO PCR Cloning Kit (Invitrogen), and verified by sequencing at the University of Houston Core Facility. A BLAST search was performed to identify the closest matching database sequence. A range of 10-fold serially diluted plasmid standard DNA (5×10^8 to 500 copies) was run on each qPCR plate in triplicate. Standard curve R^2 values were calculated for standards. Copies per gram of stool were calculated, accounting for initial sample DNA concentrations and stool weights. The change in bacterial levels ($\Delta\log_{10}$ copies/g stool) from entry level to each available successive timepoint was determined for each participant and median changes were calculated.

Shotgun metagenomics

Shotgun metagenomic sequencing was carried out at the University of Houston Sequencing and Gene Editing core (Houston, TX, USA) using the Nextera DNA Flex Library Prep Kit (Illumina catalogue number 20018705) for DNA library preparation and an Illumina NextSeq 500 platform for sequencing. A paired-end sequencing approach with a targeted read length of 150 bp and an insert size of 550 bp was conducted. CLC Genomics Workbench version 12 (Qiagen) was used for metagenomics assembly and analysis. Analyses were performed using the tutorial Taxonomic Profiling of Whole Shotgun Metagenomic Data, updated 6 February 2019 and available on the Qiagen website (http://resources.qiagenbioinformatics.com/tutorials/Taxonomic_Profiling.pdf). In the multiday, ascending dose study (Part 3), differential abundance plots and bacterial phylum and family level diversity were calculated to compare subjects that received either dose of ibezapolstat compared with vancomycin.

Statistical analysis

The ITT analysis plan included all subjects who received at least one dose of study medications. Sample size calculations were based on historical Phase 1 studies of antibiotics with first-in-human dosing. No inferential statistics were planned for safety or pharmacokinetic evaluations. For microbiome analysis, log-normalized qPCR data were analysed over time using repeated measures analysis to determine differences between treatment groups. Differences in β-diversity and abundance between treatment groups were assessed using PERMANOVA analysis. CLC Genomics software version 12.0.3 (Qiagen) or SAS version 9.4 (SAS institute) was used for all analyses. $P < 0.05$ was considered significant.

Results

A total of 62 subjects aged 31 ± 7 years (45% female) with an average BMI of 25 ± 3 kg/m^2 were randomized and entered the study (Figure 1); 32 subjects were in Part 1, 8 in Part 2 and 22 in Part 3. A minority of subjects were of Hispanic or Latino ethnicity (10%), with black/African American (56%) or white (39%) most common. All subjects were omnivores and no subject smoked. The study started on 26 November 2018 and the last subject had their final study follow-up on 10 May 2019. Subject demographics were comparable among all groups (Table S1, available as Supplementary data at *JAC* Online). All 62 subjects completed the study in full and were included in all evaluations.

Safety

In general, ibezapolstat was well tolerated with a safety signal similar to placebo. There were no safety signals related to physical examination or vitals (blood pressure, pulse or oral temperature) in any part of the study. No diarrhoea was reported and all stool samples were categorized as type 4 or below on the Bristol Stool Chart (formed or semi-formed). No significant abnormalities developed in the 12-lead ECG traces for any subject at any dose given. No changes were observed in serum biochemistry or haematological blood evaluations.

Proportions of subjects with any AE in each study period are shown in Figure 2(a). No dose-dependent increase in AEs was reported. The proportion of subjects with an AE was similar to placebo at each dosing level. Subjects given ibezapolstat in the fasting or fed state had a similar proportion of AEs. AEs described as possibly or probably related to ibezapolstat during the multiple ascending dose study are shown in Figure 2b. All AEs were considered mild or moderate and none required a change in therapy or intervention.

Pharmacokinetics in plasma

In general, ibezapolstat had minimal systemic absorption, with the majority of plasma concentrations less than 1 μg/mL. In the single ascending dose study, higher systemic concentrations

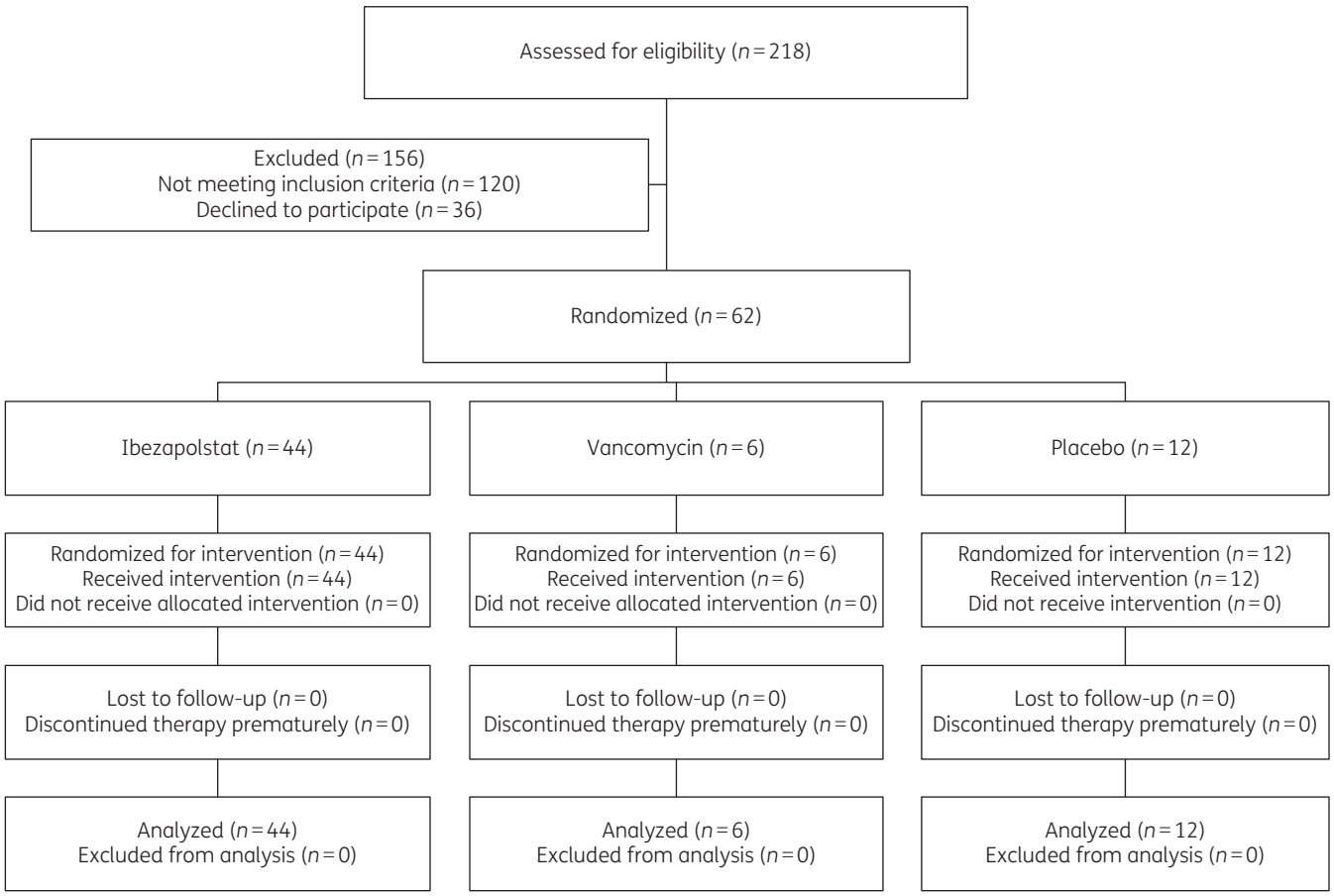

Figure 1. Study flow diagram.

(a)

Proportion of subjects with any drug-related adverse event (AE) in each study period

	Single ascending dose		Food effect	Multiple ascending dose	
Dose	*IBZ (n = 6)*	*Placebo (n = 2)*	*IBZ (n = 8)*	*IBZ (n = 6)*	*Placebo (n = 2)*
300	0%	50%	37.50%##	33%	50%
450	X	X	X	0%	0%
600	33%	50%	X	X	X
900	33%	50%	X	X	X
Summary	5 AE in 5 subjects	5 AE in 4 subjects	3 AE in 2 subjects	5 AE in 2 subjects	1 AE in 1 subject

##Fed: $n = 2$; Fast: $n = 1$; X: not tested

(b)

Description of drug-related adverse events from MAD study*

Dose (mg BID × 10 D)	AE DESCRIPTION	Number of events	INTENSITY SCALE
300	COUGH	1	MILD (GRADE 1)
300	CYSTITIS-NON INFECTIVE	1	MILD (GRADE 1)
300	DIZZINESS	1	MILD (GRADE 1)
300	EPIGASTRIC PAIN*	1	MILD (GRADE 1)
300	HEADACHE*	3	MILD (GRADE 1)
300	HEADACHE	1	MODERATE (GRADE 2)*
300	NASAL CONGESTION	1	MILD (GRADE 1)
300	TWITCHING SENSATION	1	MILD (GRADE 1)
450	DYSPEPSIA*	1	MILD (GRADE 1)
450	NAUSEA*	1	MILD (GRADE 1)
450	PROLONGED PR INTERVAL*	1	MILD (GRADE 1)
450	SHORTNESS OF BREATH*	1	MILD (GRADE 1)
450	TACHYCARDIA*	1	MILD (GRADE 1)

*Possibly or probably related
No AE required a change in therapy or intervention
AEs in placebo group ($n = 2$): Headache, rash, left hand ecchymosis

Figure 2. Adverse events in subjects receiving ibezapolstat (IBZ) or placebo. (a) Proportion of subjects with any drug-related AE in each study period. (b) Description of drug-related AEs from multiple, ascending dose study.

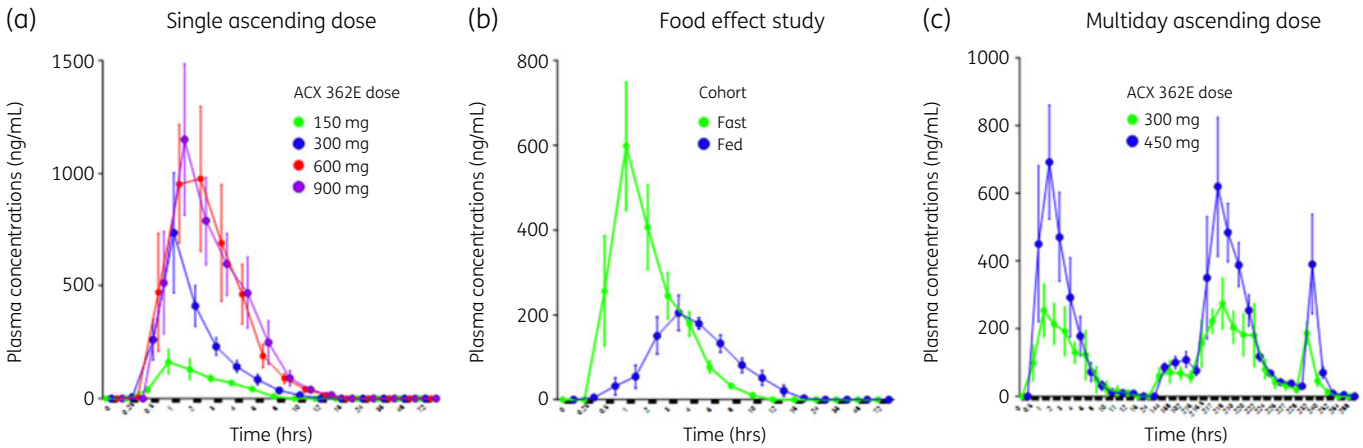

Figure 3. Ibezapolstat plasma pharmacokinetics. (a) Single ascending dose study. (b) Food effect study. (c) Multiday ascending dose study.

were observed with increased dose. However, peak plasma concentrations were less than 1 μg/mL for all doses except 900 mg (Figure 3a). Higher peak concentrations were also observed in the fast versus food-effect study. Peak concentrations were approximately 0.6 μg/mL in the fed state and 0.2 μg/mL when given with food. The rate and extent of plasma exposure were both decreased in the presence of food: C_{max} decreased by approximately 60% while the decrease in AUC was less pronounced, of approximately 20%. Food was also associated with a delay in C_{max} of approximately 2 h (Figure 3b). A similar, dose-dependent effect was observed in the multiple ascending dose study (Figure 3c). Peak concentrations were approximately 0.7 μg/mL in the 450 mg dose arm and 0.25–0.3 μg/mL in the 300 mg arm. Peak concentrations were observed approximately 2 h after the study dose.

Faecal concentrations of ibezapolstat

Dose-dependent concentrations of ibezapolstat were observed in the single-dose and multiday, ascending dose study arms (Figure 4a and c). Fasted subjects had higher ibezapolstat faecal concentrations than fed subjects, but both concentrations exceeded 500 μg/g stool by Day 2 (Figure 4b). Based on the results of the single-dose studies, two doses were studied in the multiday, ascending dose studies (300 and 450 mg). In the multiday,

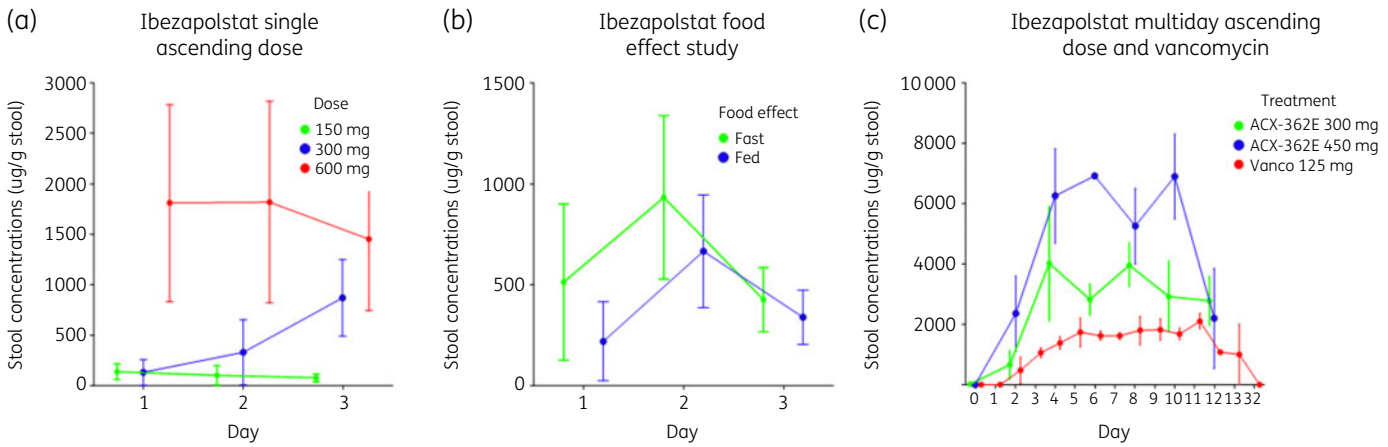

Figure 4. Ibezapolstat faecal concentrations. (a) Ibezapolstat single ascending dose study. (b) Ibezapolstat food-effect study. (c) Ibezapolstat multi-day ascending dose and vancomycin study.

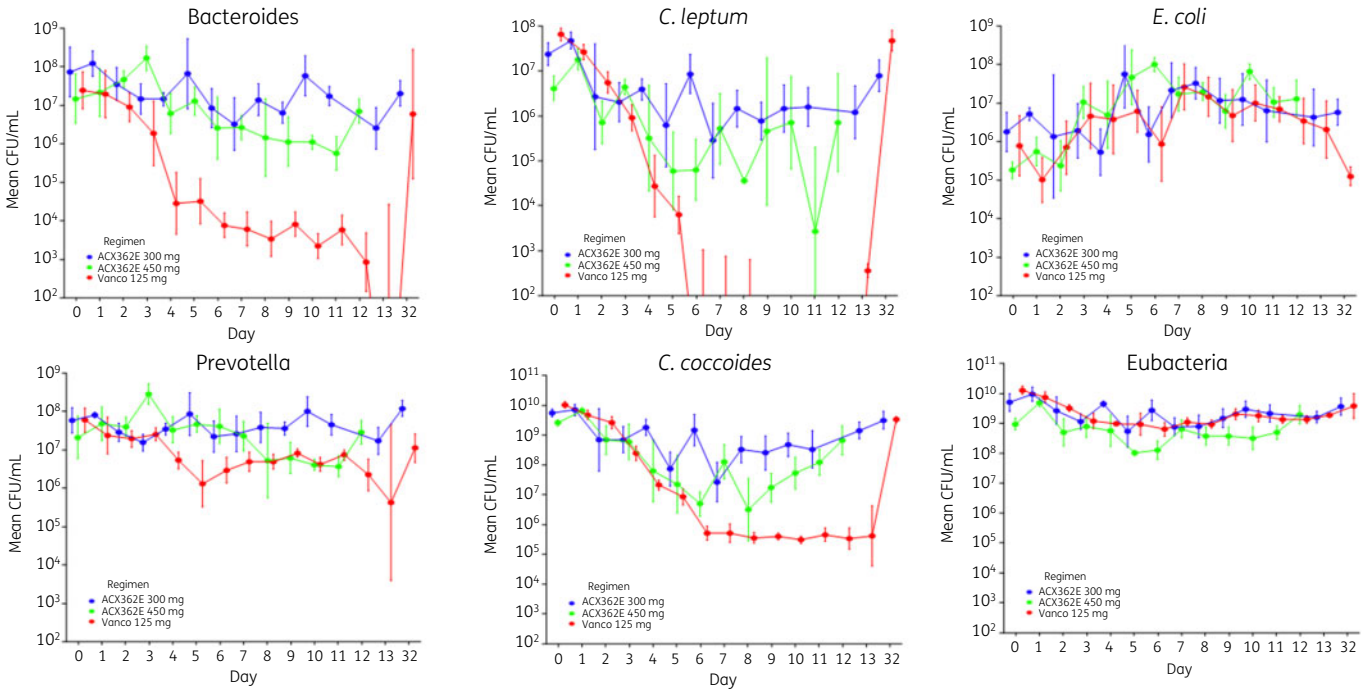

Figure 5. Microbiota levels belonging to different taxonomic groups measured by qPCR.

ascending dose study, ibezapolstat concentrations exceeded vancomycin concentrations by Day 2 and remained above 2000 μg/g of stool for the dosing time period. Maximum concentrations of 6000–7000 μg/g stool were observed for the 450 mg dose arm. By Day 2 of therapy, stool concentrations averaged 652 μg/g stool for subjects given 300 mg and 2353 μg/g stool in subjects given 450 mg (Table S2).

Microbiome analysis

In the multiday, multiple dose arm, baseline microbiota was comparable between subjects that received ibezapolstat 300 or 450 mg compared with vancomycin. Taxonomic groups measured by qPCR were similar on Day 0 but a rapid decline in *Bacteroides*, *Clostridium leptum* and *Clostridium coccoides* was observed for vancomycin but not for either ibezapolstat group (Figure 5). Differential abundance analysis and β-diversity demonstrated a distinct difference between the microbiome in subjects given vancomycin compared with either dose of ibezapolstat (Figure 6; $P = 0.006$). α-Diversity at the phylum level demonstrated an increase in the Actinobacteria phylum in subjects that received ibezapolstat and an increase in Proteobacteria in subjects given vancomycin (Figure 7a). At the family level, changes in α-diversity were due to an increase in Bifidobacteriaceae in subjects given ibezapolstat and Enterobacteriaceae or Lactobacillaceae in subjects given vancomycin (Figure 7b).

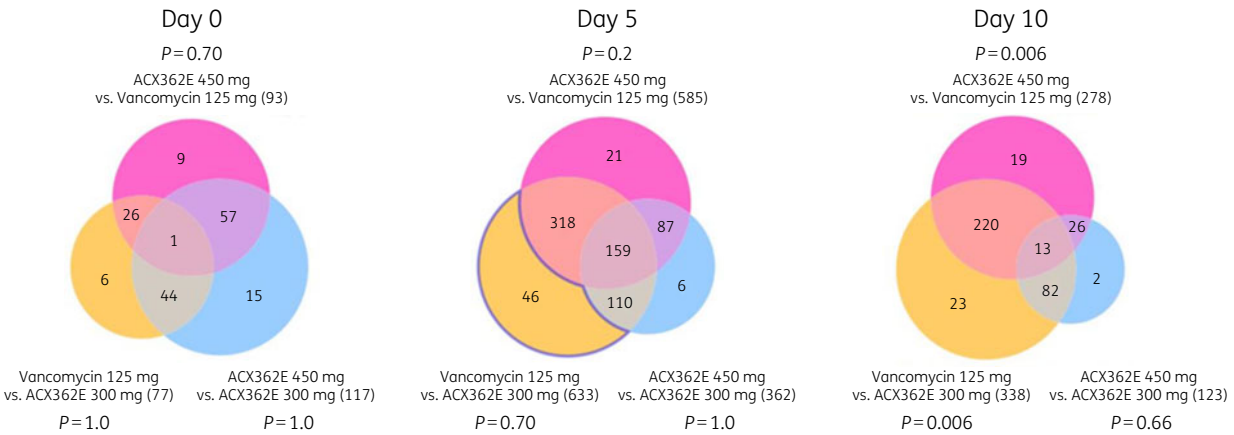

Figure 6. Metagenomic differential abundance analysis. The analysis was performed using a generalized linear model differential abundance test on samples defined by treatment type. The Wald test was used to determine significance between group pairs.

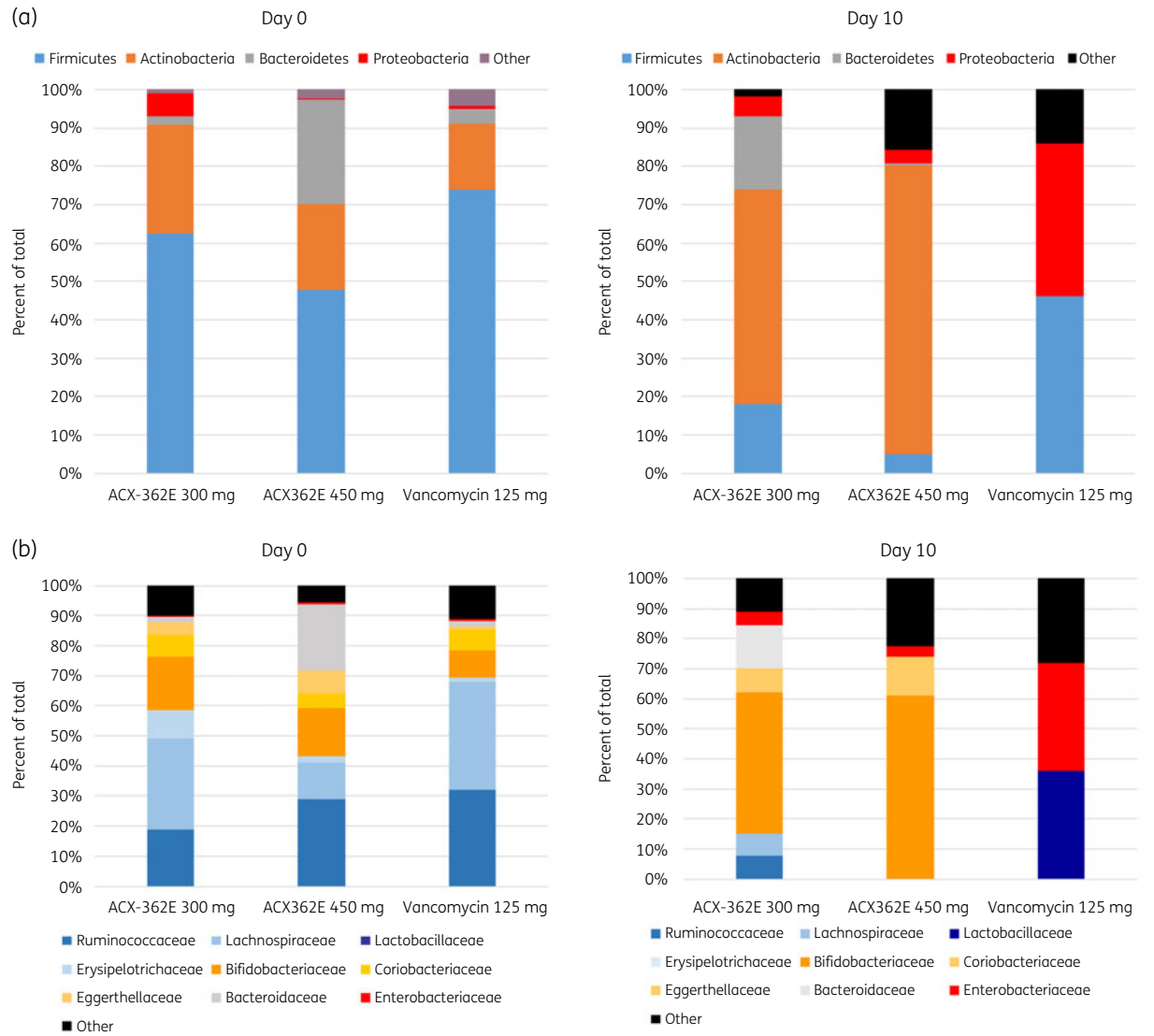

Figure 7. (a) Phylum-level diversity in subjects given ibezapolstat versus vancomycin. (b) Family-level diversity in subjects given ibezapolstat versus vancomycin.

Discussion

CDI is the leading healthcare-associated infection in the USA, with more than 450000 cases annually.[15] Despite a high disease burden and significant mortality and morbidity, guideline-recommended antibiotic treatment options are limited by associated AEs or development of resistance.[6–8] Thus, there is an urgent need to develop new antimicrobials with unique mechanisms of action directed against *C. difficile*. Ibezapolstat is a novel DNA polymerase IIIC inhibitor with a unique mechanism of action compared with other antimicrobials directed against *C. difficile*. The DNA polymerase IIIC is essential for low G + C Gram-positive organisms, including *C. difficile*, providing a narrow spectrum of activity that would predict lower CDI recurrence rates.

This Phase 1, first-in-human study was designed to assess the safety, tolerability and pharmacokinetics of orally administered ibezapolstat. No safety signals were observed in the single-dose ascending or multiple-dose ascending trials, with AEs similar to placebo. All AEs thought to be possibly or probably related to study drug were considered mild to moderate and none required discontinuation of therapy or an active intervention. No clinically significant abnormality was identified from chemistry or haematological, ECG or physical examinations.

Oral administration was associated with minimal systemic absorption of ibezapolstat at all dosing ranges, a desirable pharmacological property for *C. difficile* antibiotics as it indicates a concentration of the active drug at the site of infection in the gut. Decreased ibezapolstat systemic concentrations were noted when subjects were given study drug with food; however, this is not likely clinically significant given the intraluminal site of action. No systemic accumulation of ibezapolstat was observed in the multiple-dose studies using 300 or 450 mg of study drug. Peak concentrations were less than 1 μg/mL. Following doses of 300 or 450 mg given twice daily, average faecal concentrations were greater than 4000 μg/g of stool at Day 4 for both dosing regimens, which were higher than vancomycin concentrations.

The selective activity of ibezapolstat in targeting low-G + C Gram-positive organisms was confirmed in this study. Vancomycin caused decreased microbiome diversity of Firmicutes, Actinobacteria and Bacteroidetes with a characteristic Proteobacteria overgrowth. Ibezapolstat effectively caused decreased diversity in low-G + C organisms (Firmicutes) but increased abundance in host Actinobacteria. At the family level, an increased prevalence of Bifidobacteriaceae was noted. The family Bifidobacteriaceae is associated with early microbiome development in humans.[16] Transition from *Bifidobacterium* species to Firmicutes then progresses based on a number of host factors as the gut microbiome develops.[17] Likewise, Proteobacteria overgrowth is associated with a markedly increased risk of systemic infections with MDR Gram-negative organisms.[18] How these distinct microbiome changes reduce the likelihood of CDI recurrence compared with vancomycin can be assessed in future studies as ibezapolstat advances into Phase 2/3 studies. Currently, these results offer important insights into microbiome changes associated with differing mechanisms of action and spectrums of activity.

Conclusions

Ibezapolstat was shown to be safe and well tolerated, with minimal systemic exposure and high stool concentrations well in excess of the MIC in this first-in-human, Phase 1 clinical trial. Ibezapolstat caused a distinct microbiome profile characterized by decreased Proteobacteria overgrowth and in contrast to vancomycin. These results support further clinical development of ibezapolstat at the 300 or 450 mg dose as a safe and effective therapy for patients with CDI, and support advancing this first-in-class DNA polymerase IIIC inhibitor into efficacy trials in patients suffering from CDI.

Funding

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Transparency declarations

K.W.G. received research grant support and consultant fees from Acurx Pharmaceuticals, Merck & Co and Summit Pharmaceuticals. M.H.S. is a shareholder and paid consultant for Acurx Pharmaceuticals. M.P.D. and M.K. have received clinical trial support from Acurx Pharmaceuticals. All other authors: none to declare.

Author contributions

All authors approved the final version of the manuscript to be submitted. Additional specific contributions are detailed by author. K.W.G. contributed to the acquisition and analysis of data and the interpretation of results and drafted, reviewed and revised the manuscript. K.B. contributed to the acquisition and analysis of data and the interpretation of results and helped to draft the manuscript. C.L., A.G.-L., D.B., M.H., J.M., C.S.Y., B.V. and M.J.A. contributed to the acquisition and analysis of data and the interpretation of results. M.P.D., M.H.S. and M.K. contributed to the study design and the interpretation of results and drafted, reviewed and revised the manuscript.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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