IDSA Infectious Diseases Society of America   hivma hiv medicine association   OXFORD

Microbiological Characterization and Clinical Outcomes After Extended-Pulsed Fidaxomicin Treatment for *Clostridioides difficile* Infection in the EXTEND Study

Mark H. Wilcox,[1,2,] Oliver A. Cornely,[3,4] Benoit Guery,[5] Chris Longshaw,[6,*] Areti Georgopali,[6] Andreas Karas,[7] Gbenga Kazeem,[6] Jose Alejandro Palacios-Fabrega,[6] and Maria J. G. T. Vehreschild[4,8]

[1]Department of Microbiology, Leeds Teaching Hospitals and University of Leeds, Leeds, United Kingdom, [2]Healthcare Associated Infections Research Group, Section of Molecular Gastroenterology, Leeds Institute for Biomedical and Clinical Sciences, University of Leeds, United Kingdom, [3]Clinical Trials Centre Cologne, ZKS Köln, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany, [4]Department of Internal Medicine, University Hospital of Cologne and German Centre for Infection Research, Partner Site Bonn-Cologne, Cologne, Germany, [5]Infectious Diseases Service, Department of Medicine, University Hospital and University of Lausanne, Lausanne, Switzerland, [6]Astellas Pharma Europe Ltd., Chertsey, United Kingdom, [7]Astellas Pharma, Ltd., Chertsey, United Kingdom, [8]Medical Clinic II, University Hospital Frankfurt, Frankfurt am Main, Germany

Background. *Clostridioides* (*Clostridium*) *difficile* infection (CDI) is diagnosed using clinical signs and symptoms plus positive laboratory tests. Recurrence of CDI after treatment is common, and coinfection with other enteric pathogens may influence clinical outcomes.

Methods. We aimed to assess rates of *C difficile* positivity, by enzyme-linked immunosorbent assay (ELISA) toxin A/B and BioFire FilmArray, and the effect of enteric coinfection on clinical outcomes, using samples from the EXTEND study of extended-pulsed fidaxomicin (EPFX) versus standard vancomycin.

Results. All 356 randomized and treated patients tested positive for *C difficile* toxin A/B by local tests; a majority (225 of 356, 63.2%) also tested positive by both ELISA and BioFire. Most stool samples taken at screening tested positive for *C difficile* only using BioFire (EPFX: 112 of 165, 69.7%; vancomycin: 118 of 162, 72.8%). Of the 5 patients who failed treatment and had stool samples available, all (1) had tested negative for *C difficile* by BioFire at screening and (2) were negative by ELISA at time of treatment failure. When analyzed by BioFire results at screening, rates of sustained clinical cure at 30 days after end of treatment were numerically higher with EPFX than with vancomycin for almost all patients, except for those who tested negative for *C difficile* but positive for another pathogen. However, these outcome differences by presence of coinfection did not reach statistical significance. Whole-genome sequencing analysis determined that 20 of 26 paired samples from patients with recurrence were reinfections with the same *C difficile* strain.

Conclusions. Testing for presence of copathogens in clinical trials of antibiotics could help to explain clinical failures.

Keywords: *Clostridioides difficile*; fidaxomicin; gut microbiota; infection; vancomycin.

Clostridioides (*Clostridium*) *difficile* infection (CDI) is a major health burden in developed countries, causing approximately 20%–30% of antibiotic-associated diarrhea [1, 2]. The diagnosis of CDI is based on clinical signs and symptoms in combination with laboratory tests, such as cell cytotoxicity neutralization assay, toxigenic culture, enzyme immunoassay (EIA) detection of *C difficile* toxin A/B or glutamate dehydrogenase (GDH), and/or nucleic acid amplification tests (NAATs)

that detect toxin genes [3]. Because no single test is suitable for use as a stand-alone test for CDI, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Society for Healthcare Epidemiology of America/Infectious Diseases Society of America guidelines recommend the use of a 2-step algorithm (preferably NAAT or GDH EIA, followed by toxin A/B EIA) to diagnose CDI [3, 4]. The NAATs that detect multiple pathogens simultaneously, such as the BioFire platform [3], are also useful to explore the nature of the infection. Coinfection of *C difficile* and norovirus, for example, is associated with worse clinical symptoms and poorer outcomes than if *C difficile* alone was present [5]. Further characterization of *C difficile* isolates is possible with polymerase chain reaction (PCR) ribotyping, which can be combined with antibiotic susceptibility testing to identify isolates with reduced susceptibility to antibiotics [6].

Both initial and recurrent CDI follow the disruption and delayed recovery of normal gut microbiota, commonly as the result of antibiotic treatment [7–9]. Although vancomycin is

the standard of care for severe CDI [10], it also has a deleterious effect on the intestinal bacterial microbiota [11, 12], and approximately 24% of patients have CDI recurrence after vancomycin treatment [13]. Fidaxomicin, a narrow-spectrum macrocyclic antibiotic, has demonstrated noninferiority to vancomycin for initial clinical cure in Phase III clinical trials [14–16]. These studies also showed that fidaxomicin was associated with lower rates of recurrence compared with vancomycin (14% versus 26%) [15], thought to be the result of gut microbiota preservation [8, 11, 12]. Moreover, an in vitro human gut model demonstrated that an extended-pulsed fidaxomicin (EPFX) regimen (initial twice-daily dosing for 5 days, followed by single doses on alternate days until Day 25) may allow persistence of fidaxomicin at inhibitory concentrations for a longer time period, compared with standard dosing (twice-daily dosing for 10 days) [17]. In an in vitro gut model, this had the effect of suppressing *C difficile* while facilitating microbiota recovery [17].

The EXTEND clinical trial analyzed the efficacy of an EPFX regimen in patients with CDI aged 60 years and older [18], an age group at particular risk of CDI complications and recurrence [19]. Patients treated with EPFX had a significantly higher rate of sustained clinical cure (SCC) at 30 days after the end of treatment (EOT) and a significantly lower 90-day recurrence rate [18], compared with vancomycin. Using samples from the EXTEND study, we assessed the rates of *C difficile* toxin A/B positivity using a central laboratory enzyme-linked immunosorbent assay (ELISA) and the effect of coinfection with other enteric pathogens on clinical outcomes. We also aimed to distinguish between recurrent CDI caused by the same *C difficile* strain as the original CDI episode (relapse) or with a different *C difficile* strain (reinfection), using whole-genome sequencing (WGS).

MATERIALS AND METHODS

Study Design and Patients

EXTEND was a Phase IIIb/IV randomized, controlled, open-label, parallel-group study conducted conducted between November 5, 2014 and May 5, 2016 at 86 centers in 21 European countries. Full details of study methodology have been published previously [18]. Eligible patients included hospitalized patients ≥60 years of age with clinically confirmed CDI (defined as >3 unformed bowel movements or ≥200 mL of unformed stool for patients with rectal collection devices) in the 24 hours before randomization plus a positive local laboratory test within 48 hours of randomization for the presence of *C difficile* toxin A/B in stool. Exclusion criteria included CDI therapy for >1 day within the past 48 hours and >2 previous CDI episodes within 3 months of enrollment; at sites in Germany, patients with inflammatory bowel disease were also excluded. Institutional review boards at each site approved the study protocol and amendments, and patients provided written informed consent. EXTEND is registered with ClinicalTrials. gov, number NCT02254967.

Treatments, Assessments, and Endpoints

Patients were randomized 1:1 to receive fidaxomicin (200 mg tablets) twice daily on Days 1–5 then once daily on alternate days on Days 7–25, or vancomycin (125 mg capsules) 4 times daily on Days 1–10 (Supplementary Figure 1) [18].

The modified full analysis set (mFAS) included all randomized patients who met the inclusion criteria and received ≥1 dose of study medication. Test of cure (TOC) assessments were conducted 2 days after EOT (vancomycin, Day 12; EPFX, Day 27), comprising presence of CDI, CDI severity score, and clinical response, in line with ESCMID criteria [10]. The primary endpoint was SCC of CDI at 30 days after EOT with vancomycin (Day 40) or EPFX (Day 50). Sustained clinical cure of CDI was defined as clinical response at TOC with no subsequent CDI recurrence. Recurrence was defined as diarrhea occurring to a greater extent than the frequency recorded at TOC, positive confirmation of *C difficile* toxin A/B, and a requirement for further CDI therapy. Patients were followed up until Day 90. Stool samples were collected for all patients at screening (Day 0) and at any unscheduled visit for treatment failure or CDI recurrence.

Enzyme-Linked Immunosorbent Assay Detection of Clostridium difficile Toxin A/B in the Central Laboratory

All stool samples collected at screening and samples obtained at suspected treatment failure or recurrence were analyzed for the presence or absence of *C difficile* toxin A or B at a central laboratory (LCG, Fordham, UK), using qualitative ELISA. The result provided a semiquantitative measure of toxin concentration.

BioFire

All stool samples collected at screening and any stool sample collected at treatment failure or recurrence were analyzed at a central laboratory (LCG) for bacterial, parasitic, or viral enteric pathogens (including *C difficile*) using a PCR-based multiplex test (BioFire FilmArray Gastrointestinal Panel; bioMérieux, Basingstoke, UK). Patients were then categorized according to the BioFire result from their stool samples collected at screening: Group 1, positive for *C difficile* only; Group 2, positive for *C difficile* and another pathogen; Group 3, negative for *C difficile* but positive for another pathogen; and Group 4, negative for all pathogens (Supplementary Table S1). Testing for additional pathogens was not performed at the study sites.

Polymerase Chain Reaction Ribotyping and Antibiotic Susceptibility Testing

Clostridium difficile isolates were stored on Amies charcoal swabs and shipped at ambient temperature to the central testing

facility (Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, UK). Polymerase chain reaction ribotyping was performed using a capillary electrophoresis-based ribotyping approach. The minimum inhibitory concentration of fidaxomicin and vancomycin was determined on the cultured strains using agar dilution methods.

Whole-Genome Sequencing

To differentiate between CDI relapse and reinfection, paired stool samples were collected at screening and from patients with recurrence after TOC. *Clostridium difficile* isolates from these paired samples underwent WGS and single nucleotide variant (SNV) difference analysis at a central laboratory (LGC Genomics, Berlin, Germany). Paired isolates with ≤2 SNVs were considered the same *C difficile* strain and defined as CDI relapse; paired isolates with >10 SNVs were considered different *C difficile* strains and defined as CDI reinfection. Paired isolates with >2 but ≤10 SNVs were defined as indeterminate.

RESULTS

Patient Characteristics

The primary results of the study have been reported in full elsewhere [18]. Of 364 randomized patients, 362 were included in the safety analysis set and 356 in the mFAS. The median age was 75 years (Supplementary Table S2). At screening, all patients tested positive for *C difficile* toxin A/B by local laboratory test.

Clostridium difficile Toxin A/B Central Laboratory Enzyme-Linked Immunosorbent Assay

A similar proportion of patients in each treatment arm (EPFX: 116 of 165, 70.3%; vancomycin: 114 of 164, 69.5%) tested positive for *C difficile* toxin A/B in stool samples by central laboratory ELISA (Table 1). Across treatment arms, the overall proportion of patients who tested positive by central ELISA decreased to 2.3% at Day 12 then increased to 15.2% at Day 55 (Table 1). However, the number of samples available for central testing was much lower at all study visits after screening. All 5 patients who experienced treatment failure tested negative for *C difficile* toxin by central ELISA, whereas the majority of patients who experienced recurrence tested positive in both EPFX (8 of 9, 88.9%) and vancomycin (18 of 25, 72.0%) treatment arms (Table 1).

BioFire Analysis

Proportion of Patients Positive for Clostridium difficile by BioFire

The majority of patients (287 of 356, 80.6%) tested positive for *C difficile* by BioFire at screening (Table 2). Almost all (225 of 230, 97.8%) samples that tested positive for *C difficile* by ELISA also tested positive for *C difficile* by BioFire (Table 2). Across treatment arms, 40 of 356 (11.2%) patients tested negative for *C difficile* by BioFire (Table 2).

Table 1. Proportion of Patients With Positive Result From Central Laboratory ELISA for *Clostridioides (Clostridium) difficile* Toxin A/B in Stool, by Study Visit (mFAS)

Visit		EPFX (N = 177)	Vancomycin (N = 179)	Total (N = 356)
Screening	n	165	164	329
	Positive, n (%)	116 (70.3)	114 (69.5)	230 (69.9)
	Negative, n (%)	49 (29.7)	50 (30.5)	99 (30.1)
Day 5	n	27	21	48
	Positive, n (%)	5 (18.5)	5 (23.8)	10 (20.8)
	Negative, n (%)	22 (81.5)	16 (76.2)	38 (79.2)
Day 12	n	24	20	44
	Positive, n (%)	1 (4.2)	0	1 (2.3)
	Negative, n (%)	23 (95.8)	20 (100.0)	43 (97.7)
Day 27	n	20	17	37
	Positive, n (%)	1 (5.0)	3 (17.6)	4 (10.8)
	Negative, n (%)	19 (95.0)	14 (82.4)	33 (89.2)
Day 40	n	21	16	37
	Positive, n (%)	1 (4.8)	3 (18.8)	4 (10.8)
	Negative, n (%)	20 (95.2)	13 (81.3)	33 (89.2)
Day 55	n	19	14	33
	Positive, n (%)	3 (15.8)	2 (14.3)	5 (15.2)
	Negative, n (%)	16 (84.2)	12 (85.7)	28 (84.8)
Treatment failure (unscheduled visit)	n	2	3	5
	Positive, n (%)	0	0	0
	Negative, n (%)	2 (100.0)	3 (100.0)	5 (100.0)
Recurrence (unscheduled visit)	n	9	25	34
	Positive, n (%)	8 (88.9)	18 (72.0)	26 (76.5)
	Negative, n (%)	1 (11.1)	7 (28.0)	8 (23.5)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EPFX, extended-pulsed fidaxomicin; mFAS, modified full analysis set (all randomized patients with positive local laboratory test for *C difficile* toxin A/B at screening, who received at least 1 dose of study medication).

Proportion of Patients Positive for Other Enteric Pathogens

BioFire analysis revealed a range of enteric pathogens in stool samples (mFAS) taken at screening, including *C difficile* toxin A/B in 87.8% of patients (Supplementary Table S3). However, the majority of patients in both the EPFX (112 of 165, 69.7%) and vancomycin (118 of 162, 72.8%) treatment arms tested positive for *C difficile* only (BioFire Group 1) (Supplementary Table S1, Figure 1). For the group of patients who went on to experience CDI recurrence, the majority of stool samples obtained at screening also tested positive for *C difficile* only (EPFX: 7 of 9, 77.8%; vancomycin: 15 of 24, 62.5%). Among patients who later experienced treatment failure, all of the stool samples obtained at screening tested negative for *C difficile* (BioFire Groups 3 and 4) (Figure 1).

Effect of Other Enteric Pathogens on Rate of Sustained Clinical Cure

In the total mFAS, the primary efficacy endpoint of SCC at 30 days after EOT was significantly higher in the EPFX group compared with the vancomycin group (70.1% vs 59.2%; P = .030 [Cochran-Mantel-Haenszel test]) [18]. Results of

Table 2. Number of Positive Test Results for *Clostridioides (Clostridium) difficile* at Screening From Central Laboratory ELISA Versus BioFire (mFAS)

Patient group	ELISA Result	BioFire Result			
		Positive	Negative	Missing	Total
All patients	Positive	225	4	1	230
	Negative	61	36	2	99
	Missing	1	0	26	27
	Total	287	40	29	356
EPFX	Positive	112	3	1	116
	Negative	29	20	0	49
	Missing	1	0	11	12
	Total	142	23	12	177
Vancomycin	Positive	113	1	0	114
	Negative	32	16	2	50
	Missing	0	0	15	15
	Total	145	17	17	179

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EPFX, extended-pulsed fidaxomicin; mFAS, modified full analysis set (all randomized patients with positive local laboratory test for *C difficile* toxin A/B at screening, who received at least 1 dose of study medication).

logistic regression analyses showed that the odds of achieving SCC at 30 days after EOT were 68% higher in patients treated with EPFX compared with those treated with vancomycin (Supplementary Table S4).

When analyzed by BioFire patient category, rates of SCC at 30 days after EOT were numerically higher with EPFX than with vancomycin for almost all patient categories except those who tested negative for *C difficile* but positive for another pathogen (BioFire Group 3) (Figure 2). Although there were some

differences in SCC rates at Day 30 after EOT depending on the presence or absence of non-*C difficile* enteric pathogens (Figure 2, Supplementary Table S4), these did not reach statistical significance, possibly due to small sample sizes.

Characterization of *Clostridium difficile* Isolates

Polymerase chain reaction ribotype 027 was the most prevalent ribotype detected in stool samples taken at screening (Supplementary Table S5). For the 1 patient with PCR ribotyping results available from the time of treatment failure, PCR ribotype 126 was detected. Among the 27 patients with results available from the time of recurrence, PCR ribotypes 001, 017, 126, and 176 were particularly prevalent. There was no apparent difference in *C difficile* susceptibility to fidaxomicin or vancomycin between samples taken at screening and those taken at recurrence (Supplementary Table S6).

Whole-Genome Sequencing

There was a significantly lower incidence of CDI recurrence in the EPFX arm than in the standard vancomycin arm at Days 40 (-15.1%; $P < .001$), 55 (-13.9%; $P < .001$), and 90 (-12.8%; $P < .001$). By Day 90, a total of 45 patients (EPFX, n = 11; vancomycin, n = 34) had a recurrent episode of CDI. Paired samples from baseline and from the time of recurrence were available for 26 patients. Whole-genome sequencing analysis of SNV differences showed that the most common recurrence category was reinfection (>10 SNVs), occurring in 20 of 45 (44.4%) of these patients (Table 3). Statistical analysis of CDI relapse and
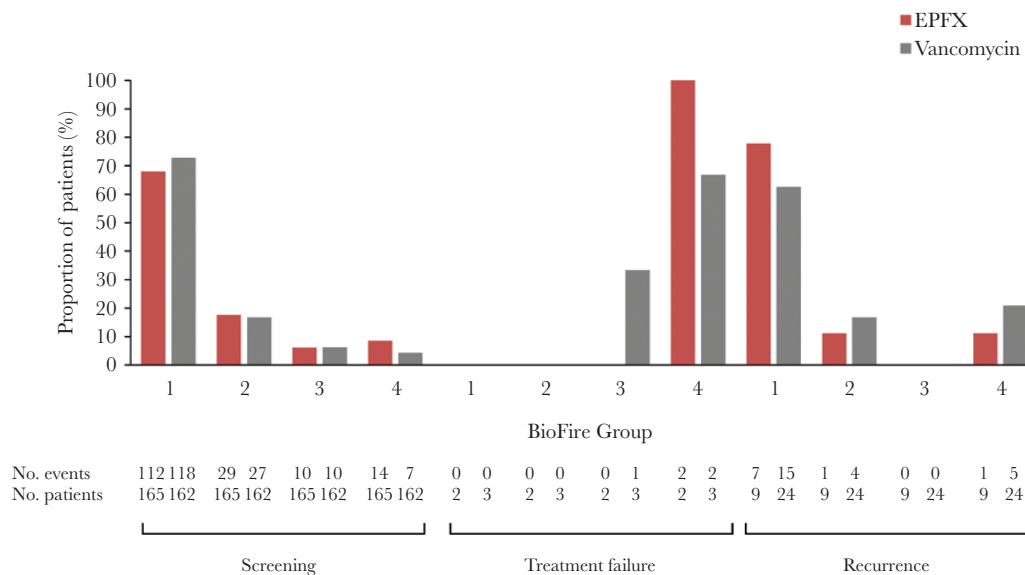

Figure 1. Proportions of patients at screening, with treatment failure and with recurrence, by BioFire categorization (mFAS). BioFire groupings were based on results at screening: Group 1, positive for *Clostridium difficile* only; Group 2, positive for *C difficile* and another pathogen; Group 3, negative for *C difficile* but positive for another pathogen; Group 4, negative for all pathogens. Percentages are calculated over the number of patients in both treatment arms at screening, who had treatment failure or who had recurrence, respectively. Data for treatment failure and recurrence are given up to Day 90. EPFX, extended-pulsed fidaxomicin; mFAS, modified full analysis set (all randomized patients with positive local laboratory test for *C difficile* toxin A/B at screening, who received at least 1 dose of study medication).
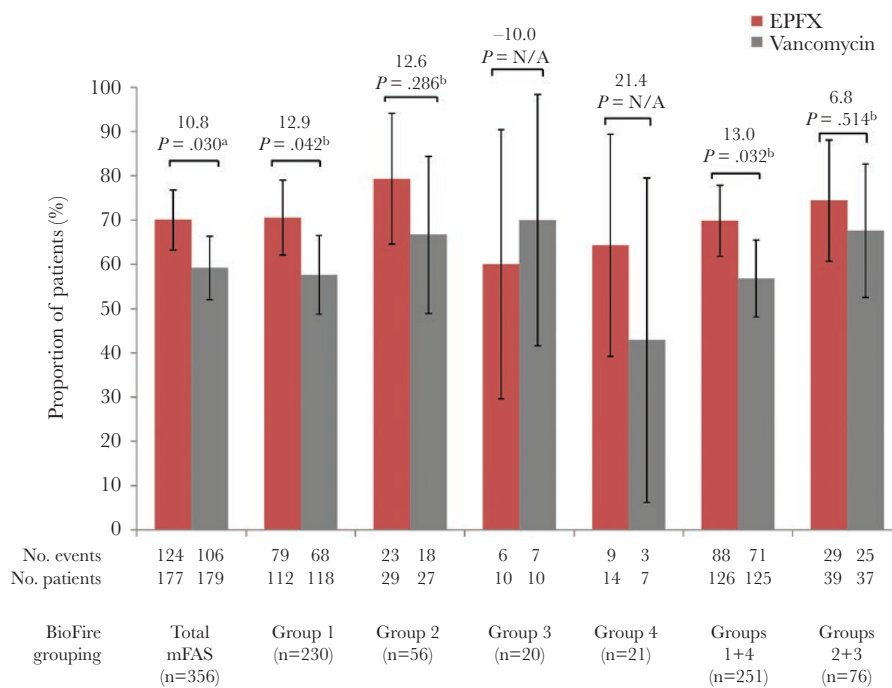
Figure 2. Sustained clinical cure of *Clostridioides* (*Clostridium*) *difficile* infection at 30 days after end of treatment by BioFire results at screening (mFAS). BioFire groups are based on results at screening: Group 1, positive for *C difficile* only; Group 2, positive for *C difficile* and another pathogen; Group 3, negative for *C difficile* but positive for another pathogen; Group 4, negative for all pathogens. [a], *P* value derived from Cochran-Mantel-Haenszel test, adjusting for baseline stratification factors. [b], *P* value derived from χ^2 test. mFAS, modified full analysis set (all randomized patients with positive local laboratory test for *C difficile* toxin A/B at screening, who received at least 1 dose of study medication); N/A, not available.

reinfection according to SNV differences was not possible owing to the small number of paired samples available.

DISCUSSION

In this secondary analysis of data from the EXTEND study, we used microbiological techniques to explore the nature of initial CDI and the effect of enteric coinfection on the rate of SCC. Although all patients in the mFAS tested positive for *C difficile* toxin A/B by local laboratory test (in line with study inclusion criteria), not all tested

positive by central laboratory ELISA or BioFire. Of 356 patients included in the mFAS, 61 tested negative by central ELISA but positive by BioFire, whereas 4 tested positive by central ELISA but negative by BioFire. A total of 225 (63.2%) patients tested positive for *C difficile* by both methods. Given that the assays have different targets, these data suggest that BioFire has a greater sensitivity for *C difficile* detection compared with toxin A/B ELISA.

The proportion of patients who tested positive for *C difficile* toxin A/B by central ELISA appeared to decrease during the course of EPFX or vancomycin treatment. It was notable that all patients who experienced treatment failure tested negative for *C difficile* toxin by central ELISA at the time of their treatment failure visit, whereas a high proportion of patients with recurrence tested positive. However, conclusions from these data are limited due to low sample numbers. Likewise, although some differences in prevalent PCR ribotypes were observed between samples taken at screening and samples from the time of recurrence, numbers were too small to draw any conclusions.

Although a range of pathogens were detected across the stool samples obtained at screening, the majority of patients (87.8%) tested positive for *C difficile* alone. A number of patients (11.2%) tested negative for *C difficile* by BioFire analysis of samples obtained at screening, despite having tested positive by local CDI tests. It is possible that this finding may account for some of the patients who experienced treatment failure: of the 5 patients who experienced treatment failure and had stool

Table 3. *Clostridioides (Clostridium) difficile* Infection Relapse and Reinfection Results at Day 90 (mFAS)

Treatment arm	EPFX	Vancomycin	Total
Patients with CDI recurrence	n = 11	n = 34	n = 45
Patients in mFAS	177	179	356
Tested pairs, n (%)[a]	7 (15.6)	19 (42.2)	26 (57.8)
Relapse (≤2 SNV)	1 (2.2)	3 (6.7)	4 (8.9)
Reinfection (>10 SNV)	5 (11.1)	15 (33.3)	20 (44.4)
Indeterminate (>2 but ≤10 SNV)	1 (2.2)	1 (2.2)	2 (4.4)
No SNV results available, n (%)	4 (8.9)	15 (33.3)	19 (42.2)

Abbreviations: CDI, *C difficile* infection; EPFX, extended-pulsed fidaxomicin; mFAS, modified full analysis set (all randomized patients with positive local laboratory test for *C difficile* toxin A/B at screening who received at least one dose of study medication); SNV, single nucleotide variant.

[a]Percentages are calculated over the total number of patients who experienced CDI recurrence in both treatment arms.

sample results available, all 5 were negative for *C difficile* at screening according to BioFire results, and all 5 tested negative for *C difficile* by central ELISA of samples taken at treatment failure. This outcome suggests that the local *C difficile* test results for some of these patients were false positives. It is also possible that the ELISA used in the central laboratory was less sensitive than that used in local laboratories, or that there was sample degradation during long-term storage before central testing could be conducted. Post hoc analysis of the primary outcome on these BioFire-determined *C difficile*-negative patients was not conducted using central ELISA-determined results, because BioFire was deemed the more sensitive test.

For the overall mFAS population, the rate of SCC at Day 30 after EOT was significantly superior in patients treated with EPFX versus vancomycin. The superior effect of EPFX was observed in all BioFire groups except for patients who tested negative for *C difficile* but positive for another pathogen. In both the EPFX and vancomycin treatment arms, numerically higher rates of SCC were observed in patients who tested positive for other pathogens in addition to *C difficile* (BioFire grouping 2 + 3), compared with those who either tested positive for *C difficile* only or were negative for all pathogens (BioFire grouping "1 + 4"). Although sample sizes were too low to reach statistical significance, the potential link between clinical outcome and BioFire result suggests that BioFire may be a useful diagnostic tool to complement local CDI testing and to identify patients most likely to respond to particular treatments.

According to analysis of SNV differences, most incidences of recurrent CDI were reinfections. This finding contrasts with previous WGS analyses of isolates from both Phase III clinical trials [20] and hospitalized patients with recurrent CDI [21], which found higher rates of relapse than reinfection. Previous clinical trial data [14, 16, 20] also demonstrated a significant improvement with fidaxomicin over vancomycin with regard to rate of relapse. However, the small number of paired samples (n = 26) in the present EXTEND study limited our ability to provide a definitive assessment of relapse versus reinfection rates. In addition, our results are not directly comparable to previous studies due to differences in study design and outcome criteria, particularly because both previous Phase III trials used the standard fidaxomicin regimen (200 mg of fidaxomicin twice daily for 10 days), whereas the EXTEND study used the EPFX regimen. In addition, patients were followed up until Day 40 in the Phase III trials [14, 16] and until Day 90 in the EXTEND study [18].

Limitations of this study include possible analysis bias because some patients remained in the hospital for CDI treatment, giving rise to the potential for increased exposure to other *C difficile* strains circulating within the hospital and an enhanced probability of reinfection rather than relapse. In addition, the BioFire results should be interpreted with caution due to small sample sizes and the possibility that pathogens may be carried

(and detected) without being the cause of the diarrhea in these patients.

CONCLUSIONS

In conclusion, the BioFire platform may prove to be a useful tool to identify other enteric pathogens that may influence clinical outcomes in *C difficile*-related studies. These analyses of microbiological data from the EXTEND trial showed higher rates of SCC at 30 days after EOT with EPFX compared with vancomycin, in all patients except for those who tested negative for *C difficile* by BioFire and positive for another pathogen. Testing for the presence of copathogens should be considered in clinical trials of antibiotics because they could help to explain clinical failures.

References

1. Bouza E. Consequences of *Clostridium difficile* infection: understanding the healthcare burden. Clin Microbiol Infect 2012; 18(Suppl 6):5–12.

2. Lessa FC, Mu Y, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med 2015; 372:825–34.

3. Crobach MJ, Planche T, Eckert C, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect 2016; 22(Suppl 4):S63–81.

4. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 2018; 66:987–94.

5. Stokely JN, Niendorf S, Taube S, et al. Prevalence of human norovirus and *Clostridium difficile* coinfections in adult hospitalized patients. Clin Epidemiol 2016; 8:253–60.

6. Freeman J, Vernon J, Pilling S, et al. The ClosER study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes, 2011-2014. Clin Microbiol Infect 2018; 24:724–31.

7. Britton RA, Young VB. Interaction between the intestinal microbiota and host in *Clostridium difficile* colonization resistance. Trends Microbiol 2012; 20:313–9.

8. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. J Infect Dis 2008; 197:435–8.

9. Reeves AE, Theriot CM, Bergin IL, et al. The interplay between microbiome dynamics and pathogen dynamics in a murine model of *Clostridium difficile* infection. Gut Microbes 2011; 2:145–58.

10. Debast SB, Bauer MP, Kuijper EJ; European Society of Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect 2014; 20(Suppl 2):1–26.

11. Isaac S, Scher JU, Djukovic A, et al. Short- and long-term effects of oral vancomycin on the human intestinal microbiota. J Antimicrob Chemother 2017; 72:128–36.

12. Louie TJ, Cannon K, Byrne B, et al. Fidaxomicin preserves the intestinal microbiome during and after treatment of *Clostridium difficile* infection (CDI) and reduces both toxin reexpression and recurrence of CDI. Clin Infect Dis 2012; 55(Suppl 2):S132–42.

13. Vardakas KZ, Polyzos KA, Patouni K, et al. Treatment failure and recurrence of *Clostridium difficile* infection following treatment with vancomycin or metronidazole: a systematic review of the evidence. Int J Antimicrob Agents 2012; 40:1–8.

14. Cornely OA, Crook DW, Esposito R, et al. Fidaxomicin versus vancomycin for infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial. Lancet Infect Dis 2012; 12: 281–9.

15. Crook DW, Walker AS, Kean Y, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection: meta-analysis of pivotal randomized controlled trials. Clin Infect Dis 2012; 55(Suppl 2):S93–103.

16. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. N Engl J Med 2011; 364:422–31.

17. Chilton CH, Crowther GS, Todhunter SL, et al. Efficacy of alternative fidaxomicin dosing regimens for treatment of simulated *Clostridium difficile* infection in an in vitro human gut model. J Antimicrob Chemother 2015; 70:2598–607.

18. Guery B, Menichetti F, Anttila VJ, et al. Extended-pulsed fidaxomicin versus vancomycin for *Clostridium difficile* infection in patients 60 years and older (EXTEND): a randomised, controlled, open-label, phase 3b/4 trial. Lancet Infect Dis 2018; 18:296–307.

19. Abou Chakra CN, Pepin J, Sirard S, Valiquette L. Risk factors for recurrence, complications and mortality in *Clostridium difficile* infection: a systematic review. PLoS One 2014; 9:e98400.

20. Eyre DW, Babakhani F, Griffiths D, et al. Whole-genome sequencing demonstrates that fidaxomicin is superior to vancomycin for preventing reinfection and relapse of infection with *Clostridium difficile*. J Infect Dis 2014; 209:1446–51.

21. Sim JH, Truong C, Minot SS, et al. Determining the cause of recurrent *Clostridium difficile* infection using whole genome sequencing. Diagn Microbiol Infect Dis 2017; 87:11–6.