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Title page

Proposed title: Pharmacokinetic analysis of an extended-pulsed fidaxomicin regimen for the treatment of *Clostridioides* (*Clostridium*) *difficile* infection in patients aged 60 years and older in the EXTEND randomised controlled trial

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Running title: Fidaxomicin pharmacokinetics in the EXTEND randomised trial

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Abstract

Background: Fidaxomicin is a recommended treatment for *Clostridioides difficile* infection (CDI) and reduces CDI recurrence incidence versus vancomycin. An extended-pulsed fidaxomicin (EPFX) regimen further reduces recurrence frequency. However, the pharmacokinetic profile of fidaxomicin in an EPFX regimen is unknown.

Objectives: To evaluate plasma and stool concentrations of fidaxomicin and its metabolite, OP-1118, after EPFX administration for CDI.

Patients and methods: In the phase 3b/4 EXTEND trial, patients aged ≥60 years with toxin-confirmed CDI were randomised to receive EPFX (oral fidaxomicin twice-daily, days 1–5; once-daily on alternate days, days 7–25). Fidaxomicin and OP-1118 concentrations were determined using post-dose plasma samples obtained on days 5 ± 1, 12 ± 1 and 25/26, and post-dose stool samples obtained on days 5 ± 1, 12 ± 1 and 26 ± 1.

Results: Fourteen patients' plasma samples were included in the pharmacokinetic analysis; 12 of these patients provided stool samples. Median (range) plasma concentrations of fidaxomicin on day 5 ± 1 and day 25/26 were 0.0252 (0.0038–0.1220) mg/L and 0.0069 (0–0.0887) mg/L, respectively, and for OP-1118, 0.0648 (0.0142–0.3250) mg/L and 0.0206 (0–0.3720) mg/L, respectively. Median (range) stool concentrations of fidaxomicin and OP-1118 on day 26 ± 1 were 272.5 (0–524) mg/kg and 280.5 (0–1120) mg/kg, respectively.

Conclusions: EPFX treatment maintained fidaxomicin stool concentrations above the MIC_{90} against *C. difficile* until day 26 ± 1. Systemic exposure to fidaxomicin and OP-1118 was low throughout and there was no evidence of accumulation in plasma or stool during treatment.

Word count: 250/250 words

ClinicalTrials.gov identifier: NCT02254967

Sir,

Clostridioides (*Clostridium*) *difficile* infection (CDI) frequently recurs after treatment, due in part to delayed colonic microbiota recovery; the microbiota becomes increasingly disrupted with repeated antibiotic treatment for recurrent episodes.[1] Vulnerable groups, such as the elderly, are at particular risk of recurrence.[2] Guideline-recommended antibiotic treatment for adults with an initial, non-severe CDI episode are vancomycin, fidaxomicin and metronidazole.[3,4] Recurrence occurs in 27.1% and 24.0% of metronidazole- and vancomycin-treated patients, respectively,[5] and in 12.7–15.4% of standard (200 mg orally twice daily for 10 days) fidaxomicin-treated patients.[6,7] Fidaxomicin is associated with greater preservation of the gut microbiome than vancomycin, which may help reduce the CDI recurrence risk.[8,9]

The length of time that colonic fidaxomicin concentrations remain above the MIC for *C. difficile* – the apparent principal factor for clinical efficacy[10] – is the rationale behind an extended-pulsed fidaxomicin (EPFX) regimen, which has demonstrated enhanced potential to prevent recurrence by improving microbiota recovery in an *in vitro* gut model.[10] In the randomised phase 3b/4 EXTEND trial in patients aged 60 years and older, an EPFX regimen significantly increased the sustained clinical cure rate of CDI 30 days after end of treatment and significantly reduced the recurrence rate up to day 90 compared with standard vancomycin.[11] Both fidaxomicin and its major active metabolite, OP-1118, demonstrate time-dependent killing of *C. difficile* strains.[12] Following oral administration of standard-regimen fidaxomicin in patients with CDI, systemic absorption is minimal and high stool concentrations are achieved,[13] which are in excess of the MIC required to inhibit the growth of 90% (MIC_{90}) of *C. difficile* isolates.[14–17] We evaluated plasma and stool concentrations of fidaxomicin and OP-1118 after EPFX administration in patients enrolled in the EXTEND trial.

This study was conducted in accordance with Good Clinical Practice, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for

87 Human Use guidelines, local regulations and the ethical principles originating in the
88 Declaration of Helsinki (**Table S1**, available as Supplementary data at *JAC Online*). Patients
89 provided informed consent prior to the initiation of any study-specific procedures. Our PK
90 assessment was a sub-study of EXTEND (registered with ClinicalTrials.gov under
91 NCT02254967), a phase 3b/4, multicentre, randomised, controlled, superiority trial to assess
92 the efficacy and safety of EPFX compared with vancomycin, undertaken between November
93 2014 and May 2016.[11] Patients were hospitalised and aged 60 years or older with clinically
94 confirmed CDI (described in Supplemetary information at *JAC Online*).

95 Patients were randomised to receive EPFX or vancomycin. PK assessments were
96 obtained from a subset of patients randomised to EPFX who consented to participate in the
97 sub-study. Fidaxomicin 200 mg film-coated tablets (Astellas Pharma Europe, Leiden,
98 Netherlands) were scheduled to be administered orally, twice daily on days 1–5 and then
99 once daily on alternate days on days 7–25 (20 doses in total). PK assessments were
100 performed on blood (plasma) and stool samples. Blood samples were scheduled for
101 collection 1–5 h after the first dose on days 5 ± 1, 12 ± 1 and 25/26. Stool samples were
102 collected on days 5 ± 1, 12 ± 1 and 26 ± 1. Detail of plasma and stool sample scheduling
103 and handling is available as Supplementary information at *JAC Online*. Plasma and stool
104 concentrations of fidaxomicin and its metabolite, OP-1118, were measured using a validated
105 liquid chromatography tandem mass spectrometry (LC-MS/MS) method (see Supplementary
106 information at *JAC* Online). The lower limit of quantification (LLOQ) for both fidaxomicin and
107 OP-1118 in plasma was 0.05 mg/L, while in stool, the LLOQ for fidaxomicin was 0.01 mg/L
108 and 0.050 mg/L for OP-1118. All treatment-emergent adverse events (TEAEs) occurring
109 between signing informed consent and end of main study (day 90), serious AEs (SAEs)
110 occurring between informed consent and 30 days after end-of-study visit, and deaths
111 occurring at any time were recorded. Vital signs were assessed on days 1, 12, 27 and at any
112 unscheduled visits. Results are presented for the PK analysis set (PKAS), which comprised
113 all patients who completed study treatment with EPFX and had at least two plasma PK

114 samples taken within the defined study visit windows (see Supplementary information at *JAC*
115 *Online*).

116 Of 183 patients assigned to EPFX in the EXTEND trial, 181 received at least one
117 dose of EPFX, and 143 completed treatment. Thirty-five patients participated in the PK sub-
118 study and of these, 14 comprised the PKAS: all 14 patients provided plasma samples, and
119 12 provided stool samples. Baseline characteristics of patients receiving EPFX in the
120 primary analysis set for efficacy (modified full analysis set; n=177) have been reported
121 elsewhere[11] (see Supplementary information at *JAC Online*).

122 Of the patients included in the PKAS, 5/14 had blood samples taken outside of the
123 planned window of 1–5 h after the preceding dose of fidaxomicin, as detailed in
124 Supplementary data at *JAC Online*. Individual plasma fidaxomicin concentrations ranged
125 from 0 to 0.175 mg/L, and there was high variability in individual plasma levels at all three
126 sampling time points (**Table 1, Figure 1**). Median levels of fidaxomicin and OP-1118
127 declined over time. Comparisons of plasma fidaxomicin and OP-1118 concentrations over
128 time at both the population level (**Table 1**) and individual patient level (**Figure 1**) showed no
129 apparent accumulation in plasma.The median OP-1118-to-fidaxomicin ratio in plasma,
130 corrected for molecular weight, remained within the same range (3.1–3.9) over time
131 (**Table 1**).

132 Median stool fidaxomicin concentration was highest on day 5 ± 1, dropping to levels
133 5- to 3-fold lower thereafter (**Table 1**). Median stool OP-1118 concentration was lower than
134 that of fidaxomicin on day 5 ± 1. As for fidaxomicin, the highest median OP-1118
135 concentration was reported on day 5 ± 1, dropping to levels 3- to 2-fold lower thereafter.
136 Comparison of stool concentrations over time showed no apparent accumulation of
137 fidaxomicin or OP-1118. The median OP-1118-to-fidaxomicin ratio in stool, corrected for
138 molecular weight, did not change in a consistent way over time (**Table 1**). Variability was
139 high and median values ranged from 0.7 to 1.4.

Two patients had stool concentrations of both fidaxomicin and OP-1118 that were below the LLOQ: one patient on day 5 and one patient on day 26. At other assessments, the stool concentrations of fidaxomicin and OP-1118 were well above the LLOQ for these patients, and there was no apparent reason for the values that were below the LLOQ. The patient with fidaxomicin and OP-1118 concentrations below the LLOQ on day 5 had no CDI recurrence. However, it is notable that the patient with fidaxomicin and OP-1118 concentrations below the LLOQ on day 26 was free of symptoms of diarrhoea for 23 days compared with a median of 80 days for patients in the extended dataset. This patient had CDI recurrence on day 33.

Of the 14 patients in the PKAS, nine reported TEAEs with eight considered to have events unrelated to EPFX treatment (see Supplementary information at *JAC Online*).

EXTEND was the first multicentre, randomised trial to show superior sustained clinical cure and reduced CDI recurrence for EPFX, compared with standard vancomycin therapy, in patients 60 years and over.[11] We show that the EPFX regimen led to minimal systemic exposure while providing high fidaxomicin and OP-1118 stool concentrations (maximum 2630 mg/kg and 1820 mg/kg, respectively). Assuming a stool density of ~1000 g/L, maximum stool concentrations of fidaxomicin were over 500-fold greater than its MIC_{90} against *C. difficile* (0.125–0.5 mg/L);[14–17] for OP-1118, maximum stool concentrations were over 200-fold greater than its MIC_{90} (8 mg/L).[15]

Fidaxomicin and OP-1118 did not accumulate in plasma or stool over the extended treatment period; indeed, there was a decrease in fidaxomicin and OP-1118 levels in plasma and stool over time, mirroring the reduction in fidaxomicin dose from day 5 ± 1 onwards. Concentrations in stool decreased to a greater extent than in plasma and there was no dose–response relationship between stool and plasma levels. Previous *in vitro* data suggest that clinical efficacy may depend upon the length of time that fidaxomicin concentrations remain above the MIC for *C. difficile*.[10,12] We found that using the EPFX regimen sustained

median stool fidaxomicin concentrations well in excess of the fidaxomicin MIC_{90} until end of treatment, despite the reduction in effective daily dose to one-quarter of that used in the standard 10-day regimen. The results of this PK sub-study are consistent with the PK profile of standard-regimen fidaxomicin measured previously.[13] Analysis of metabolite-to-parent ratios in plasma suggest that the metabolism of fidaxomicin to OP-1118 was not affected by the extended dosing scheme.

Analysis of safety data in relation to plasma fidaxomicin concentration above or below the median day 5 ± 1 level (0.0252 mg/L) did not highlight any trends with respect to incidence or types of TEAEs reported in patients with elevated fidaxomicin levels. The small sample size of 14 patients, while typical of PK studies,[18] precluded any meaningful observations or conclusions regarding safety.

In conclusion, the EPFX regimen maintained fidaxomicin stool concentrations above the MIC_{90} against *C. difficile* until day 26 ± 1, supporting the clinical efficacy findings. There was no evidence of fidaxomicin or OP-1118 accumulation in either plasma or stool, and fidaxomicin metabolism appeared unchanged by the extended dosing scheme.

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Transparency declarations

Author conflicts of interest

BG has received research grants from Astellas, Combioxin, and Fondation Santos Suarez; personal and institutional fees from Astellas Pharma, Pfizer and MSD; and non-financial support from Astellas Pharma.

AG is a full-time employee of Astellas Pharma Europe Ltd.

IM is a full-time employee of Astellas Pharma, Inc.

AK is a full-time employee of Astellas Pharma Ltd. and has patents WO2015169451 A1 and EP17167541.6 pending to Astellas Pharma Europe Ltd.

GK was, at the time of the study's conduct, a consultant statistician working on behalf of Astellas Pharma Europe Ltd.

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Author contributions

BG, AG, AK, GK and OAC contributed to the conception and design of the study. All authors contributed to the collection, interpretation and analysis of data, and preparation of the manuscript. The manuscript was reviewed, edited and approved by all authors, who vouch for the accuracy and completeness of the data obtained.

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Tables

Table 1. Concentrations of fidaxomicin and OP-1118, and metabolite-to-parent ratios[a] in plasma, 1–5 h after the first EPFX dose on days 5 ± 1, 12 ± 1 and 25/26, and in stool on days 5 ± 1, 12 ± 1 and 26 ± 1 (PKAS)

Plasma			
Drug/metabolite	**Day 5 ± 1[b] (visit 1)**	**Day 12 ± 1[b] (visit 2)**	**Day 25/26[b] (visit 3)**
Fidaxomicin (mg/L)	n=14	n=14	n=14
Mean (SD)	0.0330 (0.0330)	0.0294 (0.0496)	0.0150 (0.0242)
Median (range)	0.0252 (0.0038–0.1220)	0.0060 (0–0.1750)	0.0069 (0–0.0887)
OP-1118 (mg/L)	n=14	n=14	n=14
Mean (SD)	0.0996 (0.0907)	0.0842 (0.1106)	0.0658 (0.1105)
Median (range)	0.0648 (0.0142–0.3250)	0.0338 (0.0009–0.3390)	0.0206 (0–0.3720)
Metabolite-to-parent ratio	n=14	n=12[c]	n=11[c]
Mean (SD)	3.7 (2.3)	6.1 (7.5)	6.0 (7.6)
Median (range)	3.1 (2.0–10.9)	3.9 (1.4–28.7)	3.5 (2.3–28.5)
Stool			
Drug/metabolite	**Day 5 ± 1**	**Day 12 ± 1**	**Day 26 ± 1**
Fidaxomicin (mg/kg)	n=11	n=10	n=10
Mean (SD)	998.6 (764.1)	177.4 (145.3)	278.6 (167.8)
Median (range)	772.0 (0–2630)	141.5 (12–437)	272.5 (0–524)
OP-1118 (mg/kg)	n=11	n=10	n=10
Mean (SD)	709.5 (530.3)	292.7 (357.9)	358.6 (306.6)
Median (range)	599.0 (0–1820)	183.5 (30–1240)	280.5 (0–1120)

Metabolite-to-parent ratio	n=10[c]	n=10	n=9[c]
Mean (SD)	0.8 (0.4)	1.7 (0.8)	1.9 (2.1)
Median (range)	0.7 (0.4–1.4)	1.4 (1.0–3.6)	0.8 (0.5–7.1)

EPFX, extended-pulsed fidaxomicin; LLOQ, lower limit of quantification; n, number of patients; PKAS, pharmacokinetic analysis set; SD, standard deviation. A total of 12 patients were included in the analysis of fidaxomicin and OP-1118 in stool samples: 11 patients provided samples at day 5 ± 1, of whom 10 provided samples at day 12 ± 1 and 9 provided samples at day 26 ± 1; one additional patient provided a stool sample at day 26 ± 1 only.

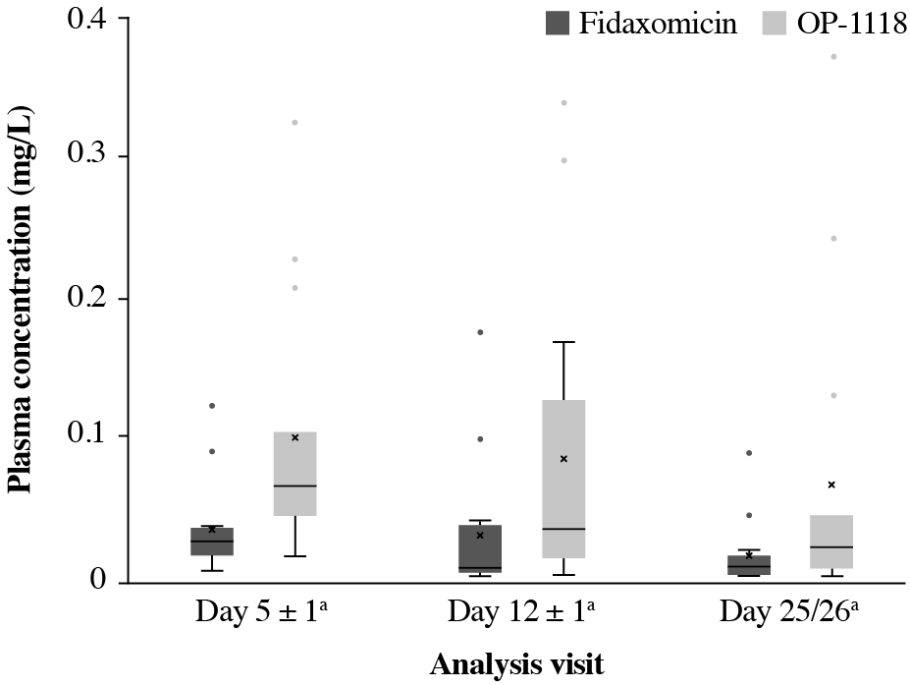
[a]Metabolite-to-parent ratio was corrected for molecular weight. [Concentration of OP-1118 × molecular weight of fidaxomicin]/[Concentration of fidaxomicin × molecular weight of OP-1118], where the molecular weight of fidaxomicin = 1058.040 g/mol and the molecular weight of OP-1118 = 987.949 g/mol.

[b]Pharmacokinetic blood sampling was scheduled for 1−5 h post dose. For patients starting EPFX in the morning on day 1 and receiving two doses of fidaxomicin on that day, blood samples were scheduled for collection on day 5 ± 1, day 11 or 13, and day 25. For patients starting in the afternoon on day 1 and receiving one dose of fidaxomicin on that day, blood samples were scheduled for collection on day 5 ± 1, day 12, and day 26. Among the patients included in the PKAS, three blood samples were taken >5 h post-dose at study visit 2, and three blood samples were taken >5 h post-dose at study visit 3.

[c]Patients with parent and/or metabolite concentrations below the LLOQ were excluded from calculations of metabolite-to-parent ratio.

Figures

Figure 1. Plasma concentrations of fidaxomicin and OP-1118 (PKAS).



PKAS, pharmacokinetic analysis set.

[a]Pharmacokinetic blood sampling was scheduled for 1−5 h post dose. For patients starting extended-pulsed fidaxomicin in the morning on day 1, blood samples were scheduled for collection on day 5 ± 1, day 11 or 13, and day 25. For patients starting in the afternoon on day 1 and receiving one dose of fidaxomicin on that day, blood samples were scheduled for collection on days 5 ± 1, day 12, and day 26. Among the patients included in the PKAS, three blood samples were taken >5 h post-dose on day 12 ± 1 (study visit 2), and three blood samples were taken >5 h post-dose on day 25/26 (study visit 3).

The length of the box represents the interquartile range (IQR; Q3 − Q1); the horizontal line in the box interior represents the median; the cross represents the mean; the whiskers represent the minimum and maximum values, excluding outliers. Lower fence = Q1 – (1.5 x IQR); upper fence = Q3 + (1.5 × IQR). Outliers are shown as dots. For OP-1118 concentrations at day 5 ± 1 and day 25/26, the upper whisker is equivalent to Q3.

Supplementary Methods

Study design and participants

Patients had clinically confirmed CDI, defined as more than three unformed bowel movements or at least 200 mL unformed stool (for patients with rectal collection devices) in the 24 h preceding enrolment, together with confirmation of the presence of *C. difficile* toxin A or B in stool within 48 h prior to enrolment. Patients were excluded if they had received therapy for CDI for more than 1 day within the previous 48 h, had more than two previous episodes of CDI within 3 months of enrolment, or had inflammatory bowel disease. All patients provided written informed consent.

Sample collection schedule and handling

The actual PK blood sampling schedule depended on whether the first dose of fidaxomicin was administered in the morning or in the afternoon on day 1. For patients starting in the morning on day 1 and receiving two doses of fidaxomicin on that day, days 12 and 27 could not be scheduled days for PK blood sampling because the patients were not taking fidaxomicin on those days. PK blood samples from these patients were therefore scheduled for day 5 ± 1, day 11 or 13 (within the allowed time window of day 12 ± 1 day), and day 25. For patients starting in the afternoon on day 1 and receiving one dose of fidaxomicin on that day, dosing was completed on day 26 and PK blood samples were therefore scheduled for days 5 ± 1, 12 and 26.

Blood samples: 3 mL of blood was collected in a Na_2 EDTA blood collection tube. The blood was centrifuged at 1500 g for 10 minutes at room temperature within 30 minutes of collection. The plasma was transferred into a 2 mL storage tube and stored at −70°C until analysis.

Stool samples: Stools were collected using a 'stool collection at home kit'. 2–3 spoonfuls of each sample were transferred into a stool container. The container was placed into a small ziplock bag and placed into a storage box, stored in a refrigerator until transfer to the site to be stored at −70°C.

Analytical methods

Plasma measurements were conducted at the bioanalytical laboratories of Astellas (Leiden, Netherlands), while stool measurements were conducted at MicroConstants (San Diego, CA, USA).

For the analysis of plasma samples, the analyte and the internal standard (IS) were extracted from 200 µL of human plasma by a combination of a protein precipitation extraction (PPE) and phospholipid removal with an Ostro plate, followed by solid phase extraction (SPE). IS working solution (25 µL, 160 ng/mL) was added to thawed plasma samples (200 µL aliquot). The samples were mixed and then subjected to SPE using an OASIS HLB plate (30 mg). The eluates resulting from extraction were evaporated to dryness under a nitrogen stream (nominal 50°C). The residues were re-dissolved in 100 µL of 40% acetonitrile and submitted for analysis by LC-MS/MS.

The samples were injected onto a Waters XSELECT CSH Phenyl-Hexyl, 2.5 µm pd, 2.1 x 50 mm Column XP (Waters, Etten-Leur, The Netherlands) and eluted with a gradient mobile phase consisting of formic acid, water and ACN. The analyte and IS were monitored on a Sciex API4000 Q Trap mass spectrometer (AB Sciex, Framingham, MA, USA) using positive Turbo Ion spray ionisation. The curve range of the method was 0.50 ng/mL to 100 ng/mL with LLOQ of 50 ng/mL for both fidaxomicin and OP-1118.

For the analysis of stool samples, weights were recorded (as raw data) and stool samples were homogenised using a multi-speed blender. The ratio of homogenisation solution (acetonitrile:acetic acid, 90:10) to stool sample was 3:1. The densities of the resulting

360 homogenates were recorded and the samples were further diluted with acetonitrile. The ratio
361 of the homogenate to acetonitrile was 1:49. The diluted stool homogenate samples were
362 stored at −20°C following sample processing.

363 Fidaxomicin, OP-1118 and the IS were extracted from 100 µL of diluted stool homogenate.
364 IS solution (0.020 mL, 1,000 ng/mL) was added to thawed, diluted stool homogenate
365 samples (100 µL aliquot) in glass test tubes. The samples were diluted with
366 water:acetonitrile (90:10), mixed and then subjected to SPE using an Oasis MAX 96 well
367 plate (10 mg). The eluates resulting from extractions were diluted with water. The residues
368 were submitted for analysis using LC-MS/MS.

369 Fidaxomicin, OP-1118 and IS were injected onto a Discovery HS PEG column (5 µm, 150 ×
370 2.1 mm, Supelco) and eluted with an isocratic mobile phase consisting of Solvent A (0.1%
371 acetic acid in water) and Solvent B (0.1% acetic acid in acetonitrile). Fidaxomicin and OP-
372 1118 were monitored on a Waters Quattro Ultima using negative electrospray ionisation. For
373 fidaxomicin, the curve range of the method was 10.0 to 2,000 ng/mL with LLOQ of
374 10.0 ng/mL. For OP-1118, the curve range of the method was 50.0 to 10,000 ng/mL with
375 LLOQ of 50.0 ng/mL.

376 Statistical analyses

377 Plasma and stool concentrations of fidaxomicin and OP-1118 were summarised by sampling
378 time point (plasma: days 5 ± 1, 12 ± 1 and 25/26; stool: days 5 ± 1, 12 ± 1 and 26 ± 1), using
379 the actual time relative to dosing. For patients with missing PK samples, decisions to
380 exclude the patient from PK analyses were made by the responsible pharmacokinetisist on a
381 case-by-case basis. Individual values below the LLOQ were set to 0 for calculation of
382 descriptive statistics, and all outliers were included in the analyses. Analyses were
383 conducted using SAS® version 9.3.

Supplementary Results

Patient characteristics

In brief, median age was 75 years, most (60%) were women, just over one-third (36%) had severe disease (defined as leucocyte count >15 × 10^9/L or rise in serum creatinine [>50% above the patient's normal levels] or albumin <30 g/L), the majority (80%) had no previous CDI occurrence in the 3 months prior to enrolment, and nearly three-quarters (72%) had taken antibiotics for a condition other than CDI in the 90 days prior to enrolment.

Plasma concentrations of fidaxomicin and OP-1118

There were 5/14 patients in the PKAS who had blood samples taken outside of the planned window of 1–5 h after the preceding dose of fidaxomicin: two patients had samples taken 26 h and 44 h post-dose at Visit 2 (days 13 and 12), respectively; one patient had a sample taken 15.5 h post-dose at Visit 2 (day 11) and 13.5 h post-dose at Visit 3 (day 25); and one patient had a sample taken 25 h post-dose at Visit 3 (day 27).

Safety

One patient had mild constipation on day 25, which was considered to be possibly related to EPFX and which resolved with treatment (**Supplementary Table S2**). Among the patients with TEAEs unrelated to EPFX, there were four with SAEs. Two patients died on days 46 and 66, respectively. Both deaths occurred in patients with fidaxomicin at or above the median plasma concentration at day 5 and neither death was considered related to study drug. The incidence and nature of adverse events did not appear to be different in patients with a fidaxomicin plasma level above or below the median concentration (0.0252 mg/L) recorded on day 5 ± 1 (**Supplementary Table S2**).

Supplementary Tables

Supplementary Table S1. IRB and Competent Authority approvals

Site	Country	IRB/Competent Authority	Approval number
Landeskrankenhaus Graz-West	Austria	Ethikkommission, der Medizinishen Universitat Vien, Borschkegasse 86/6, A-1090 Wien, Austria	1927/2014
University Hospital Motol	Czech Republic	Ethics Committee Faculty Hospital in Motol, Faculty Hospital in Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic	EK-1020/14
General Hospital of Athens G. Gennimatas AHEPA University Hospital of Thessaloniki	Greece	National Ethics Committee of Greece, 284 Mesogeion av., 15562 Athens, Greece	86/14
Orosháza Városi Önkormányzat Kórháza Semmelweis Egyetem I. Sebészeti Klinika	Hungary	Orszagos Gyogyszereszeti, Es Elelmezes-egeszsegugyi Intezet, 1051 Budapest, Zrinyi u.3, Hungary	HU36013
Szpiatal Miejski sw. Wincentego A Paulo	Poland	Komisja Etyki i Nadzoru, nad Badaniami na Ludziach i Zwierzętach, Centralnego Szpitala Klinicznego MSW, ul. Wołoska 137, 02-507 Warszawa, Poland	138/2014
Hospital Universitario Vall D'Hebron	Spain	Spanish Agency of Medicines and Health Products, C/Campezo, 1 - Edificio 8, 28022 Madrid, Spain	MUH/AEC

IRB, Institutional Review Board

Supplementary Table S2. Treatment-emergent adverse events by plasma fidaxomicin concentration level on day 5 ± 1, where patients were categorised as having a level below or above the median (0.0252 mg/L; PKAS)

Patients with adverse event, n	Patients with plasma fidaxomicin <0.0252 mg/L, n = 7	Patients with plasma fidaxomicin ≥0.0252 mg/L, n = 7
Any treatment-emergent adverse event	6[a]	3[b]
Adverse event unrelated to study treatment	6	2
Adverse event possibly related to study treatment	0	1[c]
Any serious adverse event	3[d]	1[e]
Death[f]	0	2

PKAS, pharmacokinetic analysis set.

[a]Adverse events by preferred term in n patients were: cholecystitis acute (n = 1); *Escherichia coli* urinary tract infection (n = 1); phlebitis (n = 1); cholangitis, oedema peripheral, pancreatitis, anaemia, tachycardia, proteinuria and hypoglycaemia (n = 1); ventricular extrasystoles (n = 1); and *Clostridioides* (*Clostridium*) *difficile* colitis (n = 1).

[b]Adverse events by preferred term in n patients were: death (no additional information available, n = 1); pyrexia, dyspnoea and fatal cardiopulmonary failure (n = 1); and constipation, hypokalemia, panic attack, productive cough, cough, pyelonephritis, benign prostatic hyperplasia and urinary retention (n = 1).

[c]An adverse event of mild constipation, reported on day 25 and resolved on day 28 with treatment, was considered possibly related to study drug. The dose of study treatment was not changed. This patient also had other adverse events considered unrelated to study treatment (hypokalemia, panic attack, productive cough, cough, pyelonephritis, benign prostatic hyperplasia and urinary retention).

[d]Serious adverse events by preferred term in n patients were: cholecystitis acute (n = 1); pancreatitis and hypoglycaemia (n = 1); and *C. difficile* colitis (n = 1).

[e]A serious adverse event by preferred term of cardiopulmonary failure occurred in 1 patient.

[f]All deaths were considered unrelated to study treatment: the cause of death on day 66 in an 89-year-old female patient was unknown and the cause of death on day 46 was cardiopulmonary failure in an 82-year-old female patient. Both patients had received the last dose of fidaxomicin on day 26.