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A Randomized, Placebo-controlled Trial of Fidaxomicin for Prophylaxis of *Clostridium difficile*–associated Diarrhea in Adults Undergoing Hematopoietic Stem Cell Transplantation

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**Background.** *Clostridium difficile*–associated diarrhea (CDAD) is common during hematopoietic stem-cell transplantation (HSCT) and is associated with increased morbidity and mortality. We evaluated fidaxomicin for prevention of CDAD in HSCT patients.

**Methods.** In this double-blind study, subjects undergoing HSCT with fluoroquinolone prophylaxis stratified by transplant type (autologous/allogeneic) were randomized to once-daily oral fidaxomicin (200 mg) or a matching placebo. Dosing began within 2 days of starting conditioning or fluoroquinolone prophylaxis and continued until 7 days after neutrophil engraftment or completion of fluoroquinolone prophylaxis/clinically-indicated antimicrobials for up to 40 days. The primary endpoint was CDAD incidence through 30 days after study medication. The primary endpoint analysis counted confirmed CDAD receipt of CDAD-effective medications (for any indication), and missing CDAD assessment (for any reason, including death) as failures; this composite analysis is referred to as “prophylaxis failure” to distinguish from the pre-specified sensitivity analysis, which counted only confirmed CDAD (by toxin immunosassay or nucleic acid amplification test) as failure.

**Results.** Of 611 subjects enrolled, 600 were treated and analyzed. Prophylaxis failure was similar in fidaxomicin and placebo recipients (28.6% vs 30.8%; difference 2.2% [-5.1, 9.5], *P* = .278). However, most failures were due to non-CDAD events. Confirmed CDAD was lower in fidaxomicin vs placebo recipients (4.3% vs 10.7%; difference 6.4% [2.2, 10.6], *P* = .0014). Drug-related adverse events occurred in 15.0% of fidaxomicin recipients and 20.0% of placebo recipients.

**Conclusions.** While no difference was demonstrated between arms in the primary analysis, results of the sensitivity analysis demonstrated that fidaxomicin significantly reduced the incidence of CDAD in HSCT recipients.

**Clinical Trials Registration.** NCT01691248

**Keywords.** fidaxomicin; hematopoietic stem-cell transplantation; *Clostridium difficile*–associated diarrhea; prophylaxis.

The incidence of *Clostridium difficile*–associated diarrhea (CDAD) among recipients of hematopoietic stem cell transplantation (HSCT) is 5- to 9-fold higher compared to the general hospitalized population [1–3]. Contributing risk factors include cytotoxic chemotherapy, broad-spectrum antibiotics, and, possibly, graft-versus-host disease (GVHD) [4–7]. Rates of confirmed CDAD range from 5–15% in autologous HSCT (auto-HSCT) recipients [8] and from 12–34% in allogeneic HSCT (allo-HSCT) recipients [7, 9, 10]. CDAD after allo-HSCT has been associated with higher rates of new-onset GVHD, bloodstream infections, and non-relapse mortality [4, 6, 11, 12].

Fidaxomicin at 200 mg orally twice daily is approved for the treatment of CDAD in adults, and is recommended as initial therapy for new-onset CDAD [13]. Fidaxomicin is a narrow-spectrum, macrolide antibiotic that is bactericidal against *C. difficile* in vitro [14–17], strongly inhibits the production of *C. difficile* toxins A and B [18], and has minimal effects on the gut microbiota [19]. Fidaxomicin and its major metabolite (OP-1118) are potent inhibitors of *C. difficile* spore formation [20]. In 2 randomized, controlled trials [21, 22], fidaxomicin at 200 mg twice daily was non-inferior to oral vancomycin at 125 mg 4 times daily in achieving clinical cures of CDAD, and resulted in significantly lower rates of CDAD recurrence...
(13–15% vs 25–27%). In a post hoc analysis of combined data from these 2 trials, fidaxomicin was significantly more effective than vancomycin in achieving clinical cures in the presence of concomitant antibiotic therapy and in preventing recurrence, regardless of concomitant antibiotic use [23]. Another post hoc analysis of these 2 trials showed that cancer patients who received fidaxomicin had significantly higher rates of clinical cures and sustained clinical responses than cancer patients who received vancomycin [24].

Given the increased risk for CDAD and its associated complications, prevention of CDAD may be of benefit in HSCT patients. This study examined the efficacy and safety of fidaxomicin as a prophylaxis against CDAD in patients undergoing allo- or auto-HSCT and receiving fluoroquinolone prophylaxis during neutropenia.

METHODS

Study Design and Participants

DEFLECT-1 (Protocol OPT-80-302; ClinicalTrials.gov NCT01691248) was a randomized, double-blind, placebo-controlled study conducted at 42 centers in North America and approved by the Ethical Review Committee at each study site. Individuals ≥18 years of age undergoing HSCT—including those receiving reduced-intensity (T-cell depleted) conditioning and “mini-transplants”—with planned fluoroquinolone prophylaxis during neutropenia were eligible for the study. Fluoroquinolone prophylaxis was chosen in order to standardize prophylaxis for bacterial infections, per the American Society of Blood and Bone Marrow Transplantation Guidelines [25]. Exclusion criteria included active CDAD infection (confirmed by toxin immunoassay or nucleic acid amplification tests [NAAT]) or ongoing treatment for CDAD; fulminant colitis, toxic megacolon, or ileus; receipt of a cord blood transplant; history of inflammatory bowel disease; pregnancy or breast-feeding; and current use of any drugs potentially useful in the treatment of CDAD (eg, oral vancomycin, metronidazole, oral bacitracin, fusidic acid, rifaximin, nitazoxanide). Study participants provided written informed consent before any study-related procedures were performed.

Procedures

Subjects were stratified by transplant type (autologous vs allogeneic stem cells) and randomly assigned (1:1) to receive oral fidaxomicin (200 mg) or a matching placebo once daily. Based on fecal concentration data from subjects without CDAD [26] and patients with mild to moderate CDAD [27], the fidaxomicin dose of 200 mg/day was considered likely to achieve fecal concentrations capable of suppressing C. difficile growth.

Dosing began within 2 days of start of conditioning or at fluoroquinolone initiation, whichever occurred first. The study drug was continued until 7 days after neutrophil engraftment (absolute neutrophil count [ANC] ≥ 500 cells/mm³ for 3 consecutive days or white blood cell count [WBC] >1000 cells/mm³ for 2 consecutive days); the completion of fluoroquinolone prophylaxis or any other systemic concomitant antibiotic therapy required for empiric management of febrile neutropenia or treatment of a concurrent infection during the study, whichever occurred later; or until the onset of confirmed CDAD (Supplementary Figure 1). Treatment duration was not to exceed 40 days, regardless of the time of engraftment or cessation of any antibacterial therapy. No other drugs potentially useful in the treatment of CDAD (eg, oral vancomycin, metronidazole) were allowed during the trial. Subjects requiring these medications for any reason were discontinued from study treatment.

Subjects were managed per institutional guidelines. Subjects were evaluated for CDAD symptoms twice weekly during study drug treatment, followed by twice-weekly telephone contacts through 30 days and then weekly telephone contacts through 60 days post-treatment. If CDAD was suspected, a stool sample was assayed for the presence of C. difficile based on the standard of care at the study site (either toxin immunoassay or NAAT). Subjects with confirmed CDAD during the treatment period or during the follow-up period were placed on standard of care treatment per local guidelines for CDAD management. Subjects were followed through 30 days post-treatment for all adverse events (AEs) and through 60 days post-treatment for serious AEs. Safety was assessed through laboratory evaluations (hematology, biochemistry, and urinalysis), vital signs (blood pressure, heart rate, and temperature), and physical examinations.

Statistical Analysis

Primary Efficacy Endpoint

The primary efficacy outcome was the incidence of CDAD from the first dose of study drug through 30 days after the last dose of study drug. Confirmed CDAD was defined as diarrhea (>3 unformed bowel movements in 24 hours) and a positive test for the presence of C. difficile (either by toxin immunoassay or NAAT).

Secondary Efficacy Endpoints

CDAD incidence was also evaluated at 2 secondary time points: through 60 days after the last dose of the study drug, and through 70 days after the first dose of the study drug. Time to onset of CDAD was an exploratory outcome.

Safety outcomes included treatment-emergent AEs, all-cause mortality, gastrointestinal hemorrhagic events, time to neutrophil engraftment (time elapsed from date of neutropenia [ANC < 500 cells/mm³] to the first of 3 consecutive days with ANC ≥500 cells/mm³ or the first of 2 consecutive days with WBC ≥1000 cells/mm³), and acute GVHD.
Efficacy analyses used the modified Intent-to-treat (mITT) Analysis Set, defined as all randomized subjects undergoing HSCT who received ≥1 dose of a study drug, with subjects included in the treatment group to which they were randomized. The Safety Analysis Set included all randomized subjects who received ≥1 dose of a study drug and had ≥1 post-dose safety assessment, with subjects grouped by the treatment actually received. SAS version 9.3 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

Primary and Sensitivity Analyses, Taking into Account Missing Efficacy Variables

The primary analysis classified the following outcomes as prophylaxis failure: (1) confirmed CDAD, (2) use of antibiotics potentially effective against CDAD (eg, metronidazole) for any reason, including suspected CDAD or non-CDAD indications (because CDAD-effective antibiotics would confound the CDAD assessment), and (3) missing CDAD assessments (clinical evaluation and/or toxin or NAAT assay) due to death or AE, or for any other reason (eg, loss to follow-up, missed study visits). This composite analysis was chosen as a conservative approach for handling missing data in a Phase 3 registration trial. However, because there are many reasons for missing data that are unrelated to C. difficile (eg, mortality due to underlying cancer), a pre-specified sensitivity analysis restricted to confirmed CDAD only (ie, those cases confirmed by a toxin test or NAAT) was planned a priori in order to evaluate the incidence of CDAD independent of missing data (see Supplementary Materials Protocol Section 11.9).

A 1-sided Wald test for a difference in proportions using an unpoled estimate of variance was used to test for superiority of fidaxomicin compared to the placebo at 1-sided α = .025. A 95% confidence interval surrounding the point estimate of the difference was calculated based on the stage-wise ordering method of Tsiatis [28]. Time to onset of confirmed CDAD and time to neutrophil engraftment were analyzed by the Kaplan-Meier survival analysis method [29]. The survival curves for fidaxomicin and placebo were compared using the generalized Wilcoxon and log-rank tests.

In a post hoc analysis, baseline stool samples were assayed centrally by NAAT (Cepheid Xpert C. difficile/Epi) to determine the relationship between baseline C. difficile colonization and the occurrence of confirmed CDAD, analyzed via chi-square testing.

RESULTS

Of the 611 subjects enrolled and allocated to treatment, 600 received at least 1 dose of a study drug and were assessed for safety and efficacy (Figure 1). Study treatment was completed by 227 (75.4%) fidaxomicin recipients and 218 (72.9%) placebo recipients. The mean (±SD) duration of treatment was 22.0 (±8.61) days in the fidaxomicin group and 22.7 (±8.99) days in the placebo group. Approximately 64% of subjects in each treatment group completed study treatment and follow-up. While all subjects were required to receive prophylactic fluoroquinolone antibiotics for eligibility, 75% also received non-fluoroquinolone (and non–CDAD effective) systemic antibiotics, primarily cephalosporins (56.2%), intravenous vancomycin (52.2%), and carbapenems (18.8%), during study treatment or follow-up.

Demographic and baseline characteristics were balanced across treatment groups (Table 1). Most subjects (79.2%) were inpatients at study entry, and a majority (58.7%) received auto-HSCT. For the allo-HSCT recipients, most donors were either human leukocyte antigen–matched unrelated donors or siblings (Table 1). Myeloablative conditioning regimens were more common than non-myeloablative regimens. Reasons for HSCT were diverse, comprising 64 distinct syndromes; the most common were multiple myeloma, acute myeloid leukemia, myelodysplastic syndrome, Hodgkin’s disease, and diffuse large B-cell lymphoma.

Primary Efficacy Endpoint, Taking into Account Missing Efficacy Variables

For the primary analysis, prophylaxis failure through 30 days post-treatment occurred in 28.6% of fidaxomicin-treated patients and 30.8% of those receiving the placebo (P = .278). The majority of failures in this composite analysis were attributed to non–CDAD related events: specifically, receipt of antibiotics potentially effective against CDAD (metronidazole in most cases), missing a CDAD assessment due to death or to study discontinuation due to an AE, and missing a CDAD assessment for any other reason (Table 2). Prophylaxis failure through later time points was not significantly different between fidaxomicin and the placebo (Table 2).

Sensitivity Analysis

In the sensitivity analysis, the incidence of confirmed CDAD through 30 days post-treatment was significantly lower in fidaxomicin recipients compared with placebo recipients (4.3 vs 10.7%, respectively; P = .0014). Similarly, the incidence of confirmed CDAD was lower in the fidaxomicin group than in the placebo group through 60 days after study treatment ended (5.6 vs 10.7%, respectively; P = .0117) and through study day 70 (4.7 vs 10.7%, respectively; P = .0026). Confirmed CDAD was more common in allo-HSCT recipients than in auto-HSCT recipients in both treatment groups, and was reduced in the fidaxomicin group compared with the placebo group for both transplant types (Table 2).

Secondary Analyses

When baseline stool samples were analyzed retrospectively for colonization using NAAT, 47/456 (10.3%) were positive for colonization at baseline. Of the 47 that were colonized, 16 (35.6%) later developed confirmed CDAD; 5 (31.2%) were treated with
The incidence of CDAD over time diverged significantly between the treatment groups (Figure 2). In the placebo group, most events occurred within the first 2–3 weeks after study start, while in the fidaxomicin group there was a trend towards later occurrence.

While both toxin (enzyme-linked immunosorbent assay [ELISA]) and toxin gene (NAAT) tests were allowed for confirmation of *C. difficile*, most sites in this study used direct toxin detection. Of the 45 confirmed cases of CDAD through 30 days post-treatment, 29 were confirmed directly by toxin test and 9 were confirmed by NAAT; for the remaining 7 cases, the test method was undefined.

At least 1 treatment-emergent AE was reported by nearly all subjects in both treatment groups (Supplementary Table 2). The most frequently reported AEs were diarrhea (fidaxomicin 71.0%, placebo 73.3%), nausea (fidaxomicin 62.3%, placebo 37.0%), and vomiting (fidaxomicin 41.0%, placebo 41.0%). AEs were considered by the investigator to be at least possibly related to the study drug in 15.0% of the fidaxomicin group and 20.0% of the placebo group. Most drug-related AEs were of similar or lower frequency in the fidaxomicin group versus the placebo group (Supplementary Table 2).

Serious AEs were reported for nearly one-third of subjects (fidaxomicin 32.7%, placebo 30.7%), and were considered drug-related in 1.3% of fidaxomicin and 0.7% of placebo recipients. A total of 27 subjects died during the study: 13 (4.3%) in the fidaxomicin group and 14 (4.7%) in the placebo group. None of the deaths were considered drug-related or were attributed to *C. difficile* (Supplementary Table 3), and only 3 had developed confirmed CDAD. All-cause mortality was not significantly different between treatment groups (Pearson’s chi-square test).

The median time to neutrophil engraftment was 9 days (interquartile range [IQR], 7–13 days) in the fidaxomicin
group and 9 days (IQR, 7–12 days) in the placebo group (Supplementary Figure 2). Gastrointestinal hemorrhagic events occurred in 2.7% (8/300) of fidaxomicin recipients and 5.0% (15/300) of placebo recipients ($P = .1366$). In subjects undergoing allo-HSCT, acute GVHD occurred in 39.5% (49/124) and 41.9% (52/124) of the fidaxomicin and placebo groups, respectively ($P = .6982$).

**DISCUSSION**

Patients undergoing HSCT are at increased risk for CDAD for a variety of reasons, including chemotherapy-induced immunosuppression, mucosal barrier injuries, antimicrobial prophylaxis, and antibiotic therapy given empirically or for documented infections. This study evaluated fidaxomicin 200 mg once daily as a CDAD prophylaxis in subjects undergoing allo- or auto-HSCT and receiving fluoroquinolone antibiotics, followed to 60 days post-transplant. The rate of prophylactic failure based on a composite analysis was not significantly different between fidaxomicin and placebo. This lack of a difference between fidaxomicin and placebo was primarily due to non-CDAD events. In contrast, a pre-specified sensitivity analysis showed a significantly lower rate of confirmed CDAD at 30 and 60 days in those who received fidaxomicin prophylaxis. Fidaxomicin was generally tolerated well and did not affect all-cause mortality, time to neutrophil engraftment, or incidence of gastrointestinal hemorrhage or GVHD. The overall safety profile of fidaxomicin was similar to that of placebo.

In our study population, 58.7% of subjects received auto-HSCT and 41.3% received allo-HSCT. For both transplant types, the incidence of confirmed CDAD was lower in the fidaxomicin group than in the placebo group at 30 days post-treatment (6.4 vs 14.6% after allo-HSCT; 2.8 vs 8.0% after auto-HSCT). CDAD incidence among subjects who received placebo (ie, no prophylaxis) was in the lower range of previously-reported rates for CDAD in allo-HSCT patients (12–34%) and auto-HSCT patients (5–15%) [7, 9, 10].
HSCT recipients, especially allo-HSCT recipients, are a complex group, with both infectious and non-infectious factors contributing to morbidity and mortality. In this trial, 4% of patients received metronidazole for non-CDAD indications and were considered prophylaxis failures. Other factors contributing to prophylaxis failure were missing CDAD assessments due to death or discontinuation from the study based on AEs (6%) or for other reasons (12.5%) such as missed study visits, loss to follow-up, or withdrawal of consent. The imputation of these missing data as CDAD may account, in part, for the lack of significant difference in this analysis. When the more definitive and clinically-relevant endpoint of confirmed CDAD was used in the sensitivity analysis, a significant benefit for reduction of CDAD by fidaxomicin was identified.

There are no guidelines for use of antimicrobial agents to prevent CDAD in HSCT recipients, nor has this been previously studied in a large, double-blind, placebo-controlled trial. However, 2 single-center retrospective cohort studies of
vancomycin prophylaxis in allo-HSCT were recently presented [30, 31]. The first study examined CDAD incidence during inpatient admission among 105 consecutive allo-HSCT recipients; CDAD occurred in 0/50 patients who received prophylaxis with oral vancomycin (125 mg twice daily from admission until discharge) compared to 11/55 (20%) patients who received no prophylaxis (P < .001) [30]. No follow-up to evaluate occurrence of CDAD after discharge was reported. The second study evaluated the occurrence of CDAD within 1 year post-transplant in 180 allo-HSCT patients divided into 3 cohorts. Among patients with documented histories of *Clostridium difficile* infection (CDI), CDAD occurred in 2/12 (16.6%) who received vancomycin prophylaxis vs 1/7 (14.3%) who did not receive vancomycin prophylaxis. The remaining 161 patients had no history of CDI and did not receive prophylactic vancomycin; CDAD occurred in 17 (10.6%) of this cohort [31].

A potential limitation of this study is that only 64% of subjects completed study treatment and follow-up. However, due to the high morbidity associated with HSCT, discontinuations of HSCT patients participating in clinical trials are not uncommon [32–34]. Another limitation was the lower-than-expected incidence of confirmed CDAD, which reduced the power of the study. From pre-study site surveys, we estimated a 20% incidence of confirmed CDAD for the placebo arm in the sample size calculation; however, the overall incidence observed was 10.7%. This difference from our expectations was dominated by the low incidence (8.0%) in the auto-HSCT stratum, which comprised over half of the enrolled population. Nonetheless, our study is 1 of a few large, randomized, double-blind, placebo-controlled studies evaluating prevention of CDAD in a well-defined, high-risk population.

For each subject with a new onset of diarrhea, either NAAT or direct toxin (eg, ELISA) testing was allowed for confirmation of CDAD during the treatment and follow-up periods. There has been concern voiced over the potential for over-diagnosis of CDAD in the context of NAAT assays, versus the opposite concern for under-diagnosis with less-sensitive ELISA-based methods [35]. At least 29/46 (63%) of CDAD cases were confirmed using direct toxin detection. It is worth noting that the patients in this study were diagnosed and managed for CDAD using the standard of care at each individual site. Thus, from a resource utilization perspective, the distinction between toxin-confirmed and gene-confirmed CDAD did not change site-specific clinical management.

The persistent morbidity and occasional mortality associated with CDAD among HSCT recipients warrants modalities to prevent CDAD. Except for antimicrobial stewardship and infection control measures, few methods have consistently shown benefits for reducing the incidence of CDAD among high-risk patients. The necessity of chemotherapy, systemic antibiotics, and other drugs that predispose patients to CDAD presents challenges for the modification of risk factors in HSCT patients.

The unmet medical need for a mechanism to prevent CDAD in HSCT recipients prompted this clinical investigation. Based on the results of this study, prophylaxis of CDAD with fidaxomicin can reduce the incidence of confirmed CDAD in the HSCT population. Patients with a history of CDAD or *C. difficile* colonization prior to transplantation or who are at risk for recurrent CDAD after transplantation, especially, may be suitable candidates for fidaxomicin prophylaxis. Further prophylactic studies designed around these specific high-risk patients are needed.

**Supplementary Data**

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

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**References**


