A first-in-human, randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study to assess the safety and tolerability of LFF571 in healthy volunteers

Lillian Ting1, Jens Praestgaard2, Nicole Grunenberg3,4, Jenny C. Yang1,5, Jennifer A. Leeds1 and Peter Pertel1*

1 Novartis Institutes for BioMedical Research, East Hanover, NJ, Cambridge, MA, and Emeryville, CA
2 Novartis Pharmaceutical Corp, East Hanover, NJ
3 Charles River Clinical Services Northwest, Inc, Tacoma, WA
4 Current addresses 4 Fred Hutchinson Cancer Research Center, Seattle, WA and 5 Gilead, Foster City, CA

Running title: Safety and tolerability of LFF571

*Corresponding Author:
Peter Pertel
220 Massachusetts Avenue
Cambridge, MA 02139
Phone: 1-617-871-3510
Email: peter.pertel@novartis.com
Abstract

Clostridium difficile is the leading cause of hospital-acquired infectious diarrhea. LFF571 is a novel inhibitor of the prokaryotic translation elongation factor Tu (EF-Tu) and is active against a range of bacterial species, including C. difficile. This first-in-human study investigated the safety and pharmacokinetics of single and multiple ascending oral doses of LFF571 in healthy subjects. This was a randomized, double-blind, placebo-controlled, and time-lagged study. Except for one cohort, LFF571 was given with a high fat meal in all single dose cohorts (25 mg, 100 mg, 400 mg, 1000 mg). In the multiple dose cohorts (25 mg, 100 mg, 200 mg, every 6 hours for 10 days), LFF571 was given without regard to food. A total of 56 subjects completed the study, with 32 and 25 receiving single and multiple doses, respectively. There were no deaths, no serious adverse events, and no subject withdrawals due to an adverse event. The most common adverse event was diarrhea; gastrointestinal pain or distension was also noted. Diarrhea did not develop more frequently among subjects who received LFF571 compared to placebo. LFF571 had limited systemic exposure and high steady-state fecal concentrations. The highest serum concentration of LFF571 (3.2 ng/mL) was observed after the last dose in a subject receiving 200 mg every 6 hours for 10 days. LFF571 was generally safe and well-tolerated after single and multiple oral doses in healthy subjects. The minimal serum and high fecal concentrations support the further development of LFF571 for the treatment of C. difficile infections.
Introduction

_Clostridium difficile_ is a Gram-positive, anaerobic, spore-forming bacterium, and the leading cause of antibiotic-associated diarrhea (1). _C. difficile_ infection is a serious, toxin-mediated disease, commonly associated with fever, abdominal pain, diarrhea and leukocytosis. Complications include fulminant pseudomembranous colitis, toxic megacolon, bowel perforation, sepsis, and death (7). _C. difficile_ infections typically occur after therapy with antibiotics, which may provide an environment conducive to _C. difficile_ growth by disrupting the normal intestinal flora. Increasingly, however, the disease is recognized in individuals with no recent antibiotic exposure and in previously low-risk groups, such as children, pregnant women and those with irritable bowel syndrome (17). The incidence and severity of _C. difficile_ infections have been increasing over the past decade, in part due to the emergence of hypervirulent strains such as NAP1/027/B1 (13, 15). In the United States, _C. difficile_ infection-related hospitalizations have risen from 82,000 in 1996 to about 350,000 in 2008 (1), with approximately 15,000 to 20,000 deaths attributed to _C. difficile_ each year (17).

Although treatments for _C. difficile_ infections are available, recurrences are relatively frequent after initial successful therapy, especially with vancomycin or metronidazole (9). Fidaxomicin has been shown to reduce the overall recurrence rates compared with vancomycin, although no significant reduction in relapse was noted for patients infected with the NAP1/027/B1 strain (14, 16).

The macrocyclic thiopeptide LFF571 (Figure 1) is an investigational antimicrobial agent with potent activity against Gram-positive aerobic and anaerobic bacteria, including _C. difficile_ (2, 3, 5, 6, 10). LFF571 inhibits protein synthesis by binding to bacterial elongation factor Tu (EF-Tu) (4, 11). In _vitro_, the minimal inhibitory concentration of LFF571 against 90 percent of _C. difficile_ isolates tested (MIC$_{90}$) ranged from 0.25 - 0.5 µg/mL, which is more potent than vancomycin (MIC$_{90}$ 1 - 2 µg/mL) or metronidazole (MIC$_{90}$ 0.5 - 2 µg/mL) (3, 5, 6). LFF571 is also active in _vitro_ against other aerobic and anaerobic Gram-positive organisms, including _Enterococcus faecalis_, _E. faecium_, and _S. aureus_, but has moderate to weak
activity against most Gram-negative gastrointestinal tract organisms (3, 5, 6). In a hamster model of C. difficile infection, LFF571 (5 mg/kg) was more efficacious than vancomycin (20 mg/kg) and resulted in fewer disease recurrences (18). Here, we present a first-in-human study that evaluates the safety, tolerability, and pharmacokinetics of LFF571 in healthy volunteers after administration of single and multiple ascending doses.

Methods

Study design. This first-in-human study employed a randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose design to determine the safety, tolerability, and pharmacokinetics (PK) of LFF571 in healthy subjects. The study was divided into two sequential parts, with completion of the Part I single-ascending dose study prior to the start of the Part II multiple-ascending dose cohorts. Ascending doses were given after safety assessment of the previous dose level. In each cohort, eight subjects were randomized in a ratio of 3:1 to receive LFF571 (6 subjects) or placebo (2 subjects). LFF571 was supplied as capsules at dose strengths of 25 mg and 100 mg. The placebo contained pregelatinized starch, microcrystalline cellulose and magnesium stearate. The study was conducted at Charles River Clinical Services Northwest, Inc, Tacoma, WA, in accordance with the Declaration of Helsinki and Good Clinical Practice. The study protocol was approved by the Institutional Review Board (IRB) for the center. All subjects provided written informed consent.

Part I assessed four single oral dose levels (25 mg, 100 mg, 400 mg, or 1000 mg) of LFF571. After at least 10 hours of overnight fasting, subjects received a single dose of LFF571 or placebo with an FDA high-fat, high-calorie breakfast (19). After a 6-day washout from the previous dosing period, subjects in the 400 mg group received a second single dose of LFF571 (400 mg) or placebo under fasting conditions (with water alone after at least 10 hours of overnight fast) to assess food effects on absorption.

Part II assessed LFF571 (25 mg, 100 mg or 200 mg administered every six hours) for 10 days, without regard for meals. Eligible subjects were admitted to the study center,
remained domiciled for a 10-day multiple-dosing period, and were discharged on Day 11; all subjects returned on Day 15 ± 1 day for evaluation. Subjects received a total of 40 doses of LFF571 or placebo over the course of 10 days.

Subjects. Healthy male and non-pregnant female subjects 18 to 65 years of age were eligible for the study. Good health was determined by medical history, physical examination, and laboratory tests at screening. Subjects had to weigh at least 50 kg and have a body mass index (BMI) between 18 to 32 kg/m². Only females of non-child-bearing potential were allowed to enroll and all female subjects were required to have a negative pregnancy test result at screening and at baseline. Exclusion criteria included significant illness within 2 weeks prior to initial dosing; use of tobacco products in the previous three months; drug or alcohol abuse within the past 12 months; use of prescription drugs or herbal supplements within the previous 4 weeks, or over-the-counter medication (except for incidental acetaminophen), dietary supplements or vitamins within 2 weeks; participation in any clinical investigation within at least four weeks; and donation or loss of ≥400 mL of blood within at least 8 weeks.

Safety assessment. Safety assessments consisted of collecting all adverse events with their severity and relationship to study drug, physical examinations, PK monitoring, and laboratory evaluations, including monitoring for pregnancy. In Part II only, stool samples from screening and Day 10 ± 1 day were examined for the presence of occult blood by Hemoccult/HemoQuant assays. The number of bowel movements per day, time of each bowel movement, and subject-reported Bristol Stool Scale type (12) were also recorded from the first dose on Day 1 to discharge on Day 11.

PK parameters and assessment. LFF571 levels in blood and feces were assessed throughout the study to evaluate the PK of the drug. Blood was collected in Part I on Day 1 at pre-dose and 0.5 h, 1 h, 2 h, 6 h, 12 h, and 24 h post-dose; this schedule was repeated for the fasting 400 mg cohort. In Part II, blood was collected on Day 1: pre-06:00 dose (pre-first dose); 0.5 h, 1 h, 2 h, 6 h (pre-12:00 dose), 12 h (pre-18:00 dose), and 24 h (pre-06:00 dose).
post-first dose; Day 5 and 7: pre-06:00 dose (pre-first dose of the day); Day 9: pre-06:00
dose, pre-12:00 dose, and pre-18:00 dose; Day 10: pre-06:00 dose; 0.5 h, 1 h, 2 h, and 6 h
(pre-12:00 dose) post-dose; Day 11: 6 h after last dose. Blood (3 mL) was collected in a
Serum Separator (SST) Vacutainer™ tube and processed by centrifugation for 10 min at
1000 x g, 3-5°C; serum was stored below -20°C until analysis. Fecal samples were collected
and total weight recorded in Parts I and II for the entire domiciled period after administration
of the study medication. In Part I (0 – 48 h) and Part II (last 3 days of domicile), samples from
one individual over a 24-hour period were combined and diluted in Dulbecco’s phosphate
buffered saline (DPBS). Aliquots were frozen below -60°C. LFF571 levels in serum and feces
were quantified by liquid chromatography-mass spectrometry/mass spectrometry (LC-
MS/MS). The lower limit of quantification (LLOQ) using this method was 0.5 ng/mL for serum
and 100 ng/g for feces. All randomized subjects who received at least one dose of LFF571
were included in the PK analysis population. Conventional serum PK parameters (area under
the time-concentration curve from time zero to infinity [AUCinf], AUC from time zero to last
quantifiable concentration [AUClast], maximum concentration [Cmax], time to maximum
concentration [Tmax], and accumulation ratio [AUC and Cmax on Day 10 / AUC and Cmax on
Day 1]) and amount of drug recovered in feces were evaluated where possible by non-
compartmental methods using WinNonlin Professional Version 5.2® (Pharsight Corp. St.
Louis, MO). Concentrations below the LLOQ were treated as zero.

**Statistical methods.** PK parameters were summarized by descriptive statistics. To assess
food effects, fed and fasting data from the same subjects were pooled, and AUCinf, AUClast,
AUC0-t, and Cmax were analyzed by mixed model analysis of variance with fasted or fed as a
fixed effect and a compound symmetry correlation within each subject. It was recognized that
by this analysis the fed/fasted effect could be confounded with the period effect. Ratios of
geometric means between the fasted and fed conditions were calculated together with 90%
confidence intervals.

**Results**
Subject demographics

A total of 57 subjects were enrolled in the study. Thirty-two subjects were randomized and completed Part I of the study; 24 subjects received LFF571. In Part II, 25 subjects were randomized and 24 subjects completed the study; one subject (placebo group, 100 mg every 6 h) discontinued after withdrawing consent, and this subject was replaced. The demographic data for all enrolled subjects are shown in Table 1. In Parts I and II, no subject had any medical history or condition or received any concomitant medications (such as proton pump inhibitors, H2-receptor antagonists, laxatives, antacids, or any other agents that could affect gastrointestinal motility) that were deemed to impact the safety or PK analyses.

Safety and tolerability

There were no deaths or serious adverse events, and no subject discontinued study medication due to an adverse event. Eight subjects developed a total of 13 adverse events after receiving a single dose of study drug after a high fat, high calorie meal: 7 of 24 subjects (29.2%) who received LFF571 and 1 of 8 subjects (12.5%) who received placebo. Eleven of the 13 (84.6%) adverse events were mild in severity while one (7.7%) was assessed as moderate and one (7.7%) was considered severe. The severe event was an elevated lipase level (201 U/L) two days after the subject received 400 mg of LFF571. Since the subject had no associated symptoms or signs and a repeat lipase value the next day was within the normal range (27 U/L), it was felt that this represented a false positive laboratory result. The moderate adverse event was constipation, which started the same day that the subject was dosed with 400 mg LFF571 and resolved 6 days later without therapy. This subject also had mild flatulence on the day that he received LFF571. Both the constipation and flatulence were suspected to be related to study drug. Most adverse events occurred sporadically among the dosing arms. Only three adverse events were noted in more than one subject: constipation (one subject who received 100 mg of LFF571 and one who received 400 mg of LFF571), upper viral respiratory infection (one subject who received 1000 mg of LFF571 and...
one who received placebo), and cough (the same two subjects who developed the viral infections). No subject developed diarrhea.

Four subjects in the fasting, 400 mg dose cohort developed a total of four adverse events: 2 of 6 subjects (33.3%) who received LFF571 and 2 of 2 subjects (100%) who received placebo. All adverse events were mild in severity. One adverse event, headache, was noted in two subjects (one subject who received LFF571 and one who received placebo).

In Part II, 19 subjects developed a total of 47 AEs: 13 of 18 subjects (72.2%) who received LFF571 and 6 of 7 subjects (85.7%) who received placebo (Table 2). Forty-six of the 47 (97.9%) AEs were assessed as mild in severity while one (2.1%) was moderate. The moderate event was a laceration above the right eye and was not suspected to be related to study drug. Diarrhea (including frequent bowel movements) was the most common adverse event reported in Part II, and was reported in 13 subjects. Diarrhea occurred more frequently in subjects who received placebo (85.7%) than those who received 25 mg (50.0%), 100 mg (16.7%), or 200 mg (50.0%) of LFF571 every 6 hours. All episodes of diarrhea were assessed as mild. The relatively high rates of diarrhea are likely related to the use of the specific case definition based on the Bristol Stool Chart (at least one bowel movement classified as type 5, 6, or 7) and frequency of bowel movements (3 or more bowel movements in a 24-hour period regardless of type). No bowel movement was described as liquid (type 7) and 8 subjects had only a single bowel movement that was consistent with diarrhea based on the Bristol Stool Chart (types 5 or 6).

Among subjects who received LFF571, adverse events such as gastrointestinal pain or distension developed in 3 of 6 subjects (50.0%) who received 200 mg of LFF571 every 6 hours, in contrast to 1 of 6 (16.7%) and 0 who received 25 mg and 100 mg every 6 hours, respectively. However, 2 of 7 subjects (28.6%) who received placebo also developed at least one of these adverse events. Of note, the three subjects who had symptoms that lasted for 2 or more days all received 200 mg of LFF571 every 6 hours. Although higher doses of
LFF571 may be associated with a slightly higher rate and duration of gastrointestinal pain or distension than placebo, all gastrointestinal adverse events were assessed as mild and the small numbers of subjects preclude determining if any definitive association with LFF571 exists. Only two adverse events, fatigue and headache, were noted in more than one subject (Table 2).

**PK parameters of LFF571 in serum**

LFF571 had limited systemic exposure after single doses (up to 1000 mg) and multiple doses (up to 200 mg four times daily for 10 days) in healthy subjects. In Part I, a single administration of 25 mg, 100 mg, 400 mg or 1000 mg LFF571 typically led to serum concentrations that were near or below the lower limits of quantification, 0.5 ng/mL. The highest serum concentration observed in Part I was 3.17 ng/mL, and was seen in a subject who received 400 mg under fasted conditions. The ratios of the geometric least squares means (90% confidence interval) of fed to fasted LFF571 AUC_{last} and C_{max} were 0.13 (0.06 – 0.29) and 0.29 (0.18 – 0.47), respectively. Most serum PK parameters could not be calculated due to insufficient measurable concentrations of LFF571; the measureable PK parameters for the single-ascending dose cohorts are summarized in Table 3. Maximum serum concentrations were generally observed 6 hours post-dose under fed conditions and 2 hours post-dose under fasted conditions. After 25 mg and 100 mg doses, all subjects except one had serum concentrations below the LLOQ. As the dose of LFF571 increased, the number of subjects with detectable serum concentrations increased (Table 3). Concentration profiles of the 400 mg fasting cohort, which had the highest serum LFF571 exposure, are provided in Figure 2 to illustrate the minimal LFF571 concentration achieved systemically.

After 10 days of dosing, serum concentrations of LFF571 were generally at or slightly above the LLOQ (0.5 ng/mL), as reflected by the low C_{max} observed (Table 4). The highest serum concentration observed was 3.2 ng/mL, seen on Day 11 (6 hours after the last dose) in a subject who had received 200 mg every 6 hours. Most PK parameters could not be calculated due to insufficient number of measurable concentration data; measureable PK
parameters for the multiple-ascending dose cohorts for Days 1 and 10 are summarized in Table 4. Maximum serum concentrations of LFF571 were generally observed 2 hours after dosing. At the highest LFF571 dosing regimen studied (200 mg every 6 hours), the median (range) accumulation ratios were 1.46 (0.80 – 4.51) and 1.88 (0.83 – 8.69) for $C_{\text{max}}$ and AUC$_{0-6}$, respectively.

**Fecal recovery of LFF571**

In Part I, pooled fecal samples were collected for 48 hours post-dose. The recovery of LFF571 in fecal samples varied from about 10 to 30%, as estimated by dividing the amount of drug recovered in feces by the dose administered. In Part II, three 24-hour fecal samples were collected on the last three days of dosing, approximating steady-state conditions. Under these conditions, 60 – 100% of the daily dose of LFF571 was recovered within a 24-hour period. In the multiple-dosing cohorts, the median (range) fecal concentrations of LFF571 were: 340.5 µg/g (181 – 878 µg/g), 2080 µg/g (820 – 5890 µg/g), and 5410 µg/g (2200 – 8720 µg/g) for the 25 mg every 6 hours, 100 mg every 6 hours, and 200 mg every 6 hours cohorts, respectively. The high fecal recovery and low serum concentration suggest that LFF571 remains in the gastrointestinal tract after dosing.

**Discussion**

The increased prevalence and changing epidemiology of *C. difficile* infections highlights the need for more effective treatments, particularly for severe and relapsing infections. Ideal candidates for *C. difficile* therapy are orally-administered drugs that achieve high concentrations in the gastrointestinal tract, while being minimally absorbed into the systemic circulation. Drugs should show high efficacy and low relapse rates, especially for treatment of virulent strains, and side effects should be minimal. Ideally, the dosing regimen should be as convenient as possible to minimize patient treatment burden. It is also desirable that new drugs for treatment of *C. difficile* do not select for cross-resistance to available antibacterial agents, and have limited activity against normal flora in the gut, as repopulation by these organisms is thought to be important in preventing relapse (8).
LFF571 is a novel inhibitor of bacterial EF-Tu that has potent in vitro activity against *C. difficile*. This first-in-human study investigated the safety and PK parameters of LFF571 in healthy adults. The PK parameters of orally-administered single doses (up to 1000 mg) and multiple doses (up to 200 mg every 6 hours for 10 days) of LFF571 indicated that the drug was minimally absorbed, with serum concentrations close to or below the lower limit of quantification (0.5 ng/mL). The highest serum concentration observed (3.2 ng/mL) was seen in a subject receiving the maximum dose studied (200 mg for 10 days), although a limitation of defining the peak exposure (C_max and T_max) was that the collection of PK samples was limited to 0.5, 1, 2, 6, 12, and 24 hours post-dose. Due to insufficient quantifiable serum concentrations of LFF571, most PK parameters could not be evaluated. Although slightly higher serum concentrations of LFF571 were observed under fasting conditions compared to those observed under fed conditions, levels would remain low even at higher doses and the differences observed after fasting are unlikely to be clinically relevant.

High concentrations of LFF571 in feces were detected in all cohorts. After a single dose, recovery of the drug was relatively low and variable. This was likely attributable to the short fecal collection time period of 0 – 48 hours post-dose (a limitation of the study). In fact, LFF571 was not detectable in the majority of fecal samples collected during the first day (0 – 24 hours post-dose). Depending on the bowel movement habits of subjects, it is likely that the majority of the administered dose would be excreted after 48 hours. In Part II, in which multiple feces samples were collected over the last 3 days of therapy (approximating steady-state conditions), 60 – 100% of the daily dose of LFF571 was recovered within a 24-hour period. Although the possibility of first pass biliary-hepatic elimination cannot be formally ruled out, the high fecal recovery and low serum concentrations suggest that LFF571 remains in the gastrointestinal tract after dosing. This hypothesis is supported by the high molecular weight of the compound and the low membrane permeability of LFF571 observed in Caco-2 cells (lower than the low permeability marker mannitol, data not shown).

Furthermore, <0.5% of LFF571 is absorbed in rats and *C. difficile*-infected hamsters (10). In
rats, there was minimal distribution to the liver and no measurable distribution to other
tissues (data not shown) and LFF571 was excreted in feces as unchanged drug after oral
and intravenous dosing (data not shown). Accumulation of LFF571 at the site of C. difficile
infection supports its potential use for this disease. With multiple doses, fecal concentrations
of LFF571 in healthy volunteers (which ranged from a low of 181 µg/g in a subject receiving
25 mg to a high of 8720 µg/g in subject receiving 200 mg) were approximately 362 to 34800-
times the in vitro MIC_{90} of LFF571 against C. difficile.

In this study, LFF571 was generally safe and well tolerated among subjects who
received single and multiple doses. Among subjects who received a single dose of LFF571,
the overall incidence of adverse events did not seem to increase with dose level (up to 1000
mg) and did not differ from subjects who received a placebo. The adverse events that were
noted appeared to occur sporadically. Consistent with an oral antibiotic, subjects who
received higher multiple doses of the study drug may have slightly increased rates and
duration of gastrointestinal pain or distension compared to those who received placebo. The
small numbers of subjects in this study, however, preclude determining if any definitive
association with LFF571 exists. Of note, diarrhea developed frequently among subjects who
received multiple doses of both LFF571 and placebo. This most likely represented the
stringent case definition for diarrhea, which included frequent bowel movements. No bowel
movement was described as liquid. Overall, gastrointestinal adverse events were assessed
as mild in severity and no subject stopped the study drug because of an adverse event.

In conclusion, LFF571 is a novel EF-Tu inhibitor that shows potent in vitro activity
against C. difficile and accumulated to high levels in the feces of healthy adults. LFF571 was
generally safe and well-tolerated, with limited systemic exposure. Gastrointestinal adverse
events were noted, but were mild in severity and no subject stopped study drug because of
an adverse event. These findings support the future development of LFF571 as a potential
oral treatment for C. difficile infections.
Acknowledgments

Financial support was provided by Novartis for the conduct of this study and the preparation of this manuscript. The authors wish to thank Catherine Jones, PhD, of Novartis Institutes for BioMedical Research for providing writing and editorial assistance.
References


Figure Legends and Tables

Figure 1: Chemical structure of LFF571. LFF571 is a semi-synthetic thiopeptide derived from the natural product GE2270 A.

Figure 2: LFF571 serum concentrations in healthy subjects over time. LFF571 serum concentration vs time profiles for subjects in the 400 mg single dose fasting cohort (n=6). Dashed line indicates lower limit of quantification (LLOQ).
Table 1. Demographic summary for Parts I and II

<table>
<thead>
<tr>
<th></th>
<th>Part I</th>
<th></th>
<th>Part II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFF571</td>
<td>Placebo</td>
<td>Total</td>
<td>LFF571</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>n = 24</td>
<td>n = 8</td>
<td>n = 32</td>
<td>n = 18</td>
<td>n = 7</td>
</tr>
<tr>
<td>Age, years, mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(range)</td>
<td>27.2 (18-51)</td>
<td>34.0 (19-44)</td>
<td>28.9 (18-51)</td>
<td>31.5 (18-52)</td>
<td>32.7 (20-48)</td>
</tr>
<tr>
<td></td>
<td>29 (90.6)</td>
<td>17 (94.4)</td>
<td>6 (85.7)</td>
<td>23 (92.0)</td>
<td></td>
</tr>
<tr>
<td>Gender, male, n (%)</td>
<td>22 (91.7)</td>
<td>7 (87.5)</td>
<td>29 (90.6)</td>
<td>17 (94.4)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Race n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>13 (54.2)</td>
<td>4 (50.0)</td>
<td>17 (53.1)</td>
<td>9 (50.0)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Black</td>
<td>2 (8.3)</td>
<td>2 (25.0)</td>
<td>4 (12.5)</td>
<td>3 (16.7)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (4.2)</td>
<td>1 (12.5)</td>
<td>2 (6.3)</td>
<td>1 (5.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Native American</td>
<td>1 (4.2)</td>
<td>1 (12.5)</td>
<td>2 (6.3)</td>
<td>1 (5.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (29.2)</td>
<td>0 (0.0)</td>
<td>7 (21.9)</td>
<td>4 (22.2)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Weight, kg, mean</td>
<td>74.10 (55.8-99.7)</td>
<td>82.61 (62.9-99.1)</td>
<td>76.23 (55.8 – 99.7)</td>
<td>80.1 (60.4-98.7)</td>
<td>75.10 (60.4-98.7)</td>
</tr>
<tr>
<td>(range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm, mean</td>
<td>175.4 (162-185)</td>
<td>177.0 (169-186)</td>
<td>175.8 (162 – 186)</td>
<td>175.2 (160-186)</td>
<td>173.4 (161-185)</td>
</tr>
</tbody>
</table>

Table 2. Adverse events after multiple doses of LFF571 (Part II)

<table>
<thead>
<tr>
<th>LFF571 dose</th>
<th>25 mg every 6 h</th>
<th>100 mg every 6 h</th>
<th>200 mg every 6 h</th>
<th>Placebo every 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Any adverse event</td>
<td>5 (83.3)</td>
<td>3 (50.0)</td>
<td>5 (83.3)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Gastrointestinal adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal pain or distension</td>
<td>1 (16.7)</td>
<td>0</td>
<td>3 (50.0)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Constipation</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (50.0)</td>
<td>1 (16.7)</td>
<td>3 (50.0)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Other adverse events occurring in more than 1 subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>1 (14.3)</td>
</tr>
</tbody>
</table>

a Adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology. Shown are adverse event preferred terms. Gastrointestinal pain or distension includes the preferred terms abdominal discomfort, abdominal distension, abdominal pain, abdominal pain lower, dyspepsia, and gastrointestinal pain.

b Only one diarrhea adverse event is shown if a subject had more than one episode. This occurred for one subject who received 25 mg of LFF571 every 6 hours (3 separate diarrhea episodes) and for two subjects who received placebo (2 separate episodes of diarrhea for each subject).
Table 3. Summary of serum PK parameters for Part I (single dose)

<table>
<thead>
<tr>
<th></th>
<th>25 mg (fed)</th>
<th>100 mg (fed)</th>
<th>400 mg (fed)</th>
<th>400 mg (fasted)</th>
<th>1000 mg (fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>AUC\text{0–24} (ng•hr/mL)</td>
<td>N/A</td>
<td>3.09 (N/A)</td>
<td>2.93 (2.89 – 2.97)</td>
<td>13.11 (6.65 – 35.6)</td>
<td>3.81 (2.53 – 5.41)</td>
</tr>
<tr>
<td>C\text{max} (ng/mL)</td>
<td>N/A</td>
<td>0.62 (N/A)</td>
<td>0.59 (0.58 – 0.59)</td>
<td>2.24 (0.90 – 3.17)</td>
<td>0.54 (0.51 – 0.76)</td>
</tr>
<tr>
<td>T\text{max} (hr)</td>
<td>N/A</td>
<td>6.00 (N/A)</td>
<td>6.00 (6.00 – 6.03)</td>
<td>2.00 (2.00 – 6.00)</td>
<td>6.02 (6.00 – 12.00)</td>
</tr>
</tbody>
</table>

N = number of subjects with adequate serum concentrations to evaluate AUC\text{0–24} and C\text{max}.

N/A = not available due to lack of measurable concentration data. Data are presented as median (range).

Table 4. Summary of serum PK parameters for Part II (multiple dosing)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 mg every 6 h</td>
<td>100 mg every 6 h</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>AUC\text{0–6} (ng•hr/mL)</td>
<td>N/A</td>
<td>1.73 (1.24 – 2.22)</td>
</tr>
<tr>
<td>C\text{max} (ng/mL)</td>
<td>N/A</td>
<td>0.70 (0.51 – 0.89)</td>
</tr>
<tr>
<td>T\text{max} (hr)</td>
<td>N/A</td>
<td>2.00 (2.00 – 2.00)</td>
</tr>
</tbody>
</table>

N = number of subjects with adequate serum concentrations to evaluate AUC\text{0–6} and C\text{max}. Data are presented as median (range).