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REVIEW

New antibiotics in clinical trials for *Clostridium difficile*

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ABSTRACT

Introduction: There are limited number of approved therapies for *C. difficile* infections (CDIs) and new treatments are needed to decrease recurrence rates. Over the past 5 years, four novel antibiotics have been evaluated in clinical trials that offer distinct advantages over existing therapies for the treatment of CDI.

Areas covered: This article reviews the preclinical and clinical studies of cadazolid, LFF571, ridinilazole, and surotomycin. The advantages that these antibiotics may have in the treatment of CDI is compared with current therapies metronidazole, vancomycin, and fidaxomicin.

Expert commentary: The antibiotics examined have the potential to improve rates of CDI treatment without recurrence. We anticipate that one or more of these medications will be approved within five years.

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1. Introduction

Clostridium difficile, a spore-forming opportunistic bacterium of the gastrointestinal (GI) tract, is the primary known infectious etiology of nosocomial antibiotic-associated diarrhea [1]. 'C. diff' infections (CDIs) occur when ingested spores germinate in the intestines and give rise to actively dividing colonic bacteria in patients whose gut microbiota has been altered. Risk factors for an altered fecal flora include antimicrobial and antineoplastic chemotherapy, advanced age, and disease (e.g. irritable bowel syndrome) [1,2]. Opportunistic overgrowth along the bowel results in enhanced fluid excretion and diarrheal disease that can culminate in life-threatening conditions such as pseudomembranous colitis, toxic megacolon, and sepsis. Today, there are a limited number of actively used therapies for *C. difficile*. Metronidazole (MET) (Flagyl™) is the most commonly used, first-line therapy for CDI in North America (Figure 1) [1]. For serious and recurrent illness, oral vancomycin (VAN) (Vancocin™) and fidaxomicin (FID) (Dificid™) are indicated. With limited antibiotics available and the rates of *C. difficile*-associated disease climbing, new drug candidates in mid-to-late clinical stage development hold promise that additional approved therapies will soon be available.

1.1. Pathophysiology of CDI

C. difficile is a Gram-positive bacillus with reservoirs that include soil, water, and the bowels of animals. As an anaerobic species that forms heat- and acid-stable spores upon oxygen exposure, the bacterium is able to thrive under the physiological conditions of the intestinal tract. It is estimated that 1–3% of adults are carriers of *C. difficile*, most of whom became colonized at a health-care facility [3]. Enteric colonization occurs when ingested spores germinate in the colon of persons with a depleted or altered fecal microbiota [4]. Vegetative cells attach to intestinal epithelia via adhesions. Secretion of tissue-degrading enzymes (e.g. metalloproteinases) and exotoxins (e.g. cytotoxin B) effect colonocyte death and give rise to the manifestations of CDI (Table 1).

Within 72 h of a *C. difficile* infection, patients begin to manifests signs of diarrhea with unformed or watery stools [1]. Fever and leukocytosis may also be present. Symptoms typical for the early stages of a CDI are cramping and abdominal tenderness. Passage of three or more unformed stools in 24 h is a criterion used in the initial diagnosis of CDI [1]. Stool cultures and exotoxin tests are performed to confirm the infection. When pseudomembranous colitis is suspected, a colonoscopy may be performed to detect inflammation, disease severity, and accumulation of white blood cells and cellular debris in the form of yellow plaques. If a CDI condition is left untreated, colonic dilation (i.e. toxic megacolon) may cause perforation and leakage of bowel contents in the abdominal cavity. Discontinuation of an offending medication, fluid replacement, and antimicrobial chemotherapy are countermeasures to prevent the infection from escalating to this life-threatening stage [1].

Recurrence is a significant problem in treating CDI, and the risks increase with each successive episode. Initial relapse rates range from 20% to 30% after the first episode up to 60% after multiple recurrences [3]. MET and VAN are effective bactericides against dividing bacteria but are unable to eradicate the spores. Recurrence is most often a consequence of spore

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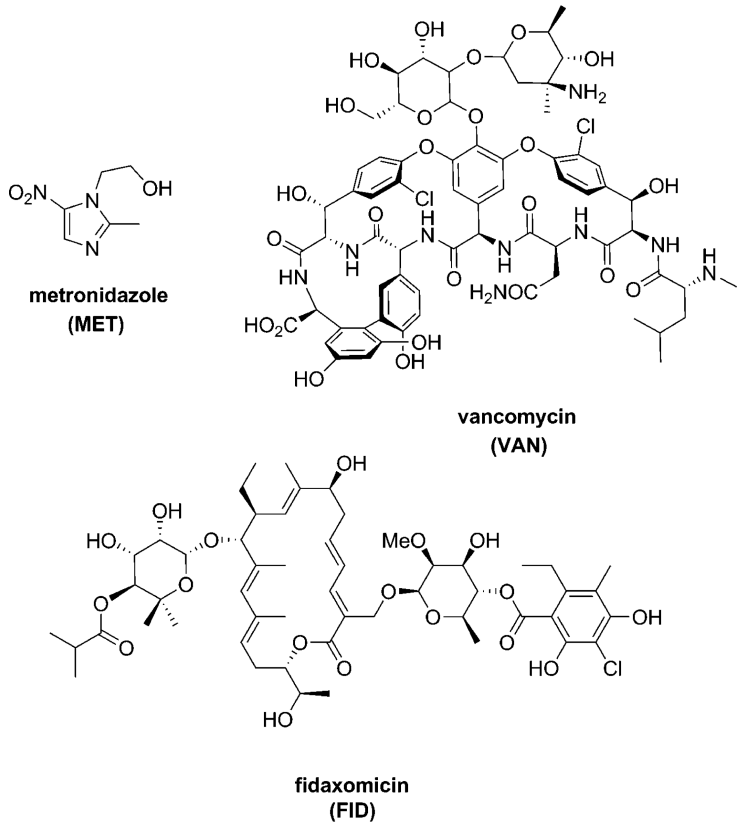

Figure 1. Treatments for *Clostridium difficile* infections (CDI): metronidazole (MW 171.2), vancomycin (MW 1449.2), and fidaxomicin (MW 1058.0).

reactivation or reexposure to the same or different strain of *C. difficile*. A noteworthy variant involved in clinical failures and fatal outbreaks is BI/NAP1/027 (i.e. ribotype 027) [4]. The markedly toxigenic and sporigenic strain is associated with high case mortality and fulminant infections in North America.

1.2. Pharmacotherapy of CDI

Anaerobic bacteria such as *Bacteroides fragilis, Prevotella* spp., and bifidobacteria are thought to play a key role in preventing the colonization and overgrowth of resistant microbes [3]. When antimicrobial therapy reduces the population of these bacteria, the opportunity for *C. difficile* to cause disease is presented. Historically, MET has been the primary treatment in patients with an initial CDI episode. Its deleterious effects on the GI anaerobic population and high relapse rates led to the introduction of low-dose VAN as an alternative therapy [1,4]. Current guidelines recommend oral VAN after repeat treatment recurrence with MET and in initial severe infections [1,4]. Alternatively, oral FID has proven to reduce recurrence rates; however, the markedly higher cost for a 10-day supply (>$3000) has relegated it as an alternative medication to MET ($35) and oral VAN ($25–$700) [5].

1.2.1. Dosage and administration

VAN and FID are the only approved antimicrobial treatments for CDI in the United States. However, oral MET is preferred in initial mild-to-moderate CDI and is given orally as 500 mg three times a day (TID) for up to 2 weeks [1,4]. Multiple daily administrations are used because MET is extensively absorbed, and low amounts are secreted back in the colon. For initial therapy of severe CDI, oral VAN is administered 125 mg four times a day (QID) for up to 14 days. Systemic side effects are rare with this regimen because oral VAN is poorly absorbed. The oral capsule ($700) can be expensive, leading many hospitals to make oral formulations from generic intravenous (IV) VAN solution ($25) [5]. Rectal instillation of VAN may also be used if complete ileus is present [1]. For initial treatment of severe, complicated infections, a regimen of oral VAN 500 mg QID and IV MET 500 mg TID is recommended. Parenteral dosing of MET which undergoes biliary elimination has the advantage of bypassing GI conditions that may impair accumulation at the target site. Alternatively, oral FID 200 mg two times a day (BID) has been shown effective for initial treatment of both non-severe and severe infections.

1.2.2. Recurrence rates

Prevention of recurrence and relapse is a main objective for effective CDI pharmacotherapy. Rates of recurrence are highly dependent on the bacterial strain involved, age, concomitant/persistent use of antibiotics for conditions other than CDI, comorbidities, and history of CDI infection [4]. Rates of recurrence following use of MET have been measured to approach as high as 40%; VAN are reportedly 20–30% [4]. A key characteristic of FID is that relapse rates tend to be lower (13–15%) [1]. To this end, the primary objectives for developing new treatments are to decrease recurrence rates and increase sustained response rates. This review will focus on four recent drug candidates in mid-to-late clinical stage development that have potential to achieve these outcomes.

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Table 1. Characteristics of *Clostridium difficile*-associated disease [1,2].

Etiology	*C. difficile*, a Gram-positive anaerobic bacilli, forms acid-, heat-, and antibiotic-stable spores
Epidemiology	Estimated 453K cases and 29K associated deaths (US 2011)
Transmission	Spore ingestion
Manifestations	Mild–severe diarrhea, pseudomembranous colitis, toxic megacolon, and sepsis
Risk factors	Hospitalization; GI surgery; ampicillin, amoxicillin, cephalosporin, fluoroquinolone, and clindamycin use; antimotility agents; advanced age (>60 years); and enteral tube feedings
Diagnosis	Positive stool culture, enzyme immunoassay detection for toxin A/B or glutamate dehydrogenase, PCR, and endoscopy
FDA-approved drugs	Vancomycin and fidaxomicin
Other treatments	Metronidazole, nitazoxanide, tigecycline, rifaximin, and fecal transplant
Prognosis	Relapse rates: 15–30%; all-cause mortality rates: 15–20%

GI: gastrointestinal; PCR: polymerase chain reaction.

2. Cadazolid (ACT-179811)

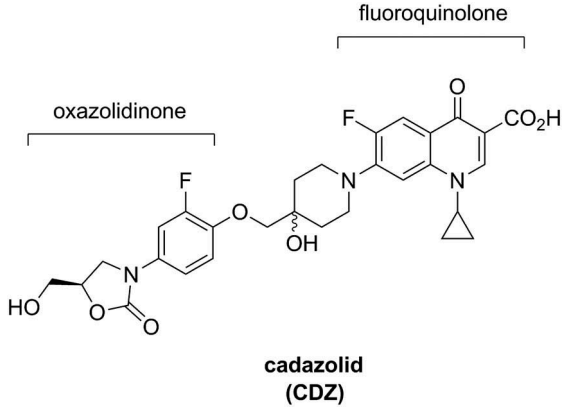
2.1. Actelion Pharmaceuticals Ltd

Cadazolid (CDZ) (ACT-179811) (Figure 2) is a novel hybrid antibiotic in development by Actelion Pharmaceuticals (Allschwil, Switzerland). Despite containing the classical fluoroquinolone (FQ) nucleus of a DNA gyrase inhibitor, the primary inhibitory effect of CDZ is on protein synthesis and is therefore classified as an oxazolidinone antibiotic [6]. The compound also displays an activity spectrum similar to the oxazolidinone linezolid (Zyvox™) and lacks the broad-spectrum profile noted for FQs. In 2014, CDZ received the Fast Track and Qualified Infectious Disease Program (QIDP) regulatory designation by the US FDA for CDI [7]. As of December 2015, Actelion Pharmaceuticals is recruiting patients for phase III trials to compare the efficacy of CDZ to VAN as a clinical cure for CDI [8].

2.2. Preclinical development

2.2.1. Microbiology

CDZ demonstrates bactericidal activity against Gram-positive and anaerobic species as disclosed in US patent 8,124,623 B2 (Table 2) [9]. Against a 133-member *C. difficile* panel, CDZ exhibited submicrogram minimum inhibitory concentrations



cadazolid
(CDZ)

Figure 2. Chemical structure of cadazolid (MW 585.6).

Table 2. Comparison of antibacterial activity of cadazolid with standard drugs [9–12].

	MIC_{90} (μg/mL)			
Species (strains)	MET	VAN	LZD	CDZ
Clostridium difficile (133)	1	1	4	0.5
C. difficile ribotype 027 (12)	1	1	2	0.125
Bacteroides fragilis (1)	0.25	8–16	1–2	2
Bifidobacterium spp. (2)	–	–	–	0.5
Eubacterium limosum (2)	–	–	–	1
Fusobacterium necrophorum (1)	–	–	–	1
Lactobacillus acidophilus (1)	–	–	–	0.5
Lactococcus lactis (1)	–	–	–	0.5
Staphylococcus aureus (26)	–	1	2	0.5
Staphylococcus epidermidis (22)	–	2	256	2
Enterococcus faecalis (11)	–	>256	16	0.5
Enterococcus faecium (20)	–	256	32	1

MET: metronidazole; VAN: vancomycin; LZD: linezolid; CDZ: cadazolid.

(MIC_{90} 0.5 μg/mL) [10]. Gram-positive cocci, including VAN-resistant enterococcus (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), were also susceptible [11]. Anaerobes and lactic acid bacteria of the gut microbiota similarly proved to be sensitive to CDZ, albeit at slightly higher drug concentrations. In an *in vitro* gut model using fecal populations obtained from healthy donors, CDZ effectively eradicated a clindamycin-induced BI/NAP1/027 infection with limited impact on the GI microflora [12]. Bifidobacteria exhibited the highest level of sensitivity while *Bacteroides fragilis* and lactobacilli counts were unaffected.

Further *in vitro* assessment revealed that CDZ is efficacious at reducing sporulation rates and inhibiting *de novo* toxin production in *C. difficile* at subgrowth inhibitory concentrations 0.25–0.5x MIC [13]. Moreover, cross-resistance with linezolid and spontaneous resistance development at 2–4x MIC after 13 passages (frequencies, $<10^{-10}$) are not observed with CDZ [6]. Time-kill kinetics studies further demonstrated that CDZ has greater bactericidal effects than VAN with a 99.9% kill rate within 24 h [6].

2.2.2. Animal studies

CDZ with a molecular weight (MW) of 585.6 has low water solubility (~150 ng/mL), possesses an acidic p*K*a (6.0), and is relatively lipophilic (log*D* 1.2) [14]. The physiochemical properties are believed to obstruct oral absorption and deliver supratherapeutic drug levels in the intestines. Oral administration studies in rats and beagles corroborated that CDZ is eliminated in feces in its parent form. In a fulminant colitis model, cure rates between Golden Syrian hamsters dosed orally at 30–100 mg/kg of CDZ were comparable to VAN at 50 mg/kg [9]. Survival rates were also assessed in a CDI mouse model [11]. Dose-dependent efficacy was observed in animals treated with 0.1–10 mg/kg for 5 days, and significant posttreatment effects were noted through day 18. Pooled survival rates were again found to be similar to VAN. In a separate study examining intestinal overgrowth of VRE, CDZ did not promote enterococcal infections in mice [15].

2.3. Clinical trials

A randomized, double-blind, placebo-controlled phase I study of CDZ in healthy subjects examined the safety, tolerability, and pharmacokinetic properties for both single- and multiple-

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ascending doses (MAD). In the single-ascending dose (SAD) arm, subjects were given either a placebo or 30, 100, 300, 1000, or 3000 mg of CDZ [14]. In the MAD arm, subjects took either a placebo or 300, 1000, or 3000 mg of CDZ BID for 10 days. A smaller phase I study investigated a single high dose of CDZ in six subjects with severe CDI in whom GI tract integrity was potentially compromised. In this single-center, open-label study, subjects were given a 3000 mg dose of CDZ after fasting [16]. The results from the two phase I trials were found to support the further development of CDZ for treatment of CDI. A phase II, multicenter, double-blind, randomized trial (NCT01222702) evaluated the safety and efficacy of CDZ with VAN as a comparator in 84 subjects. Volunteers were provided either oral VAN 125 mg QID or CDZ 250, 500, or 1000 mg BID for 10 days. Although CDZ did not meet the preset goal, the clinical cure rate was higher with all doses of CDZ (68.4–80%) than with VAN (68.2%) and progressed to phase III trial [17]. Actelion Pharmaceuticals is currently recruiting 640 CDI patients to compare the efficacy and safety of CDZ to VAN in a multicenter phase III trial (NCT01983683) [8].

2.3.1. Dosage and administration

In the trials to date, CDZ has been provided as a powder for oral suspension, to be reconstituted prior to administration. In both phase I and II trials, the powder was reconstituted with mineral water [14,18]. There has been no evidence that increasing the CDZ dose over 250 mg BID improves efficacy. The number of subjects experiencing adverse events (AEs) did not change with increasing doses. After the phase II study, it was decided to bring the 250 mg BID CDZ dose to the phase III trials [17]. Food was found to influence the rate and extent of absorption CDZ in less than dose-proportional increases.

2.3.2. Safety and tolerability

When studied in healthy subjects, CDZ was well tolerated with only mild AEs noted [16]. In the MAD arm, the most frequent AE was headache, occurring in a total of six patients in both the CDZ and placebo groups. Three headaches were reported within 2 h of receiving either placebo or CDZ. No diarrhea was reported in the MAD group [14]. When CDZ was studied in subjects with severe CDI in the second phase I trial, no clinically significant AEs were noted. Three AEs did occur (candiduria, constipation, and hypoproteinemia), but these were deemed to be unrelated to CDZ.

In the phase II trial, treatment-emergent AEs were experienced by 30%, 23%, or 30% of subjects receiving 250, 500, or 1000 mg of CDZ, respectively, and 46% in the VAN arm [17]. The majority of the AEs were mild or moderate intensity and included headache, dizziness, confusion, dyspepsia, and pruritus in the CDZ arm. Study treatment was discontinued early for two subjects receiving 1000 mg of CDZ due to unresolved CDI. Other serious AEs included pneumothorax, bronchial secretion retention, respiratory arrest, and renal failure, which all occurred in the same patient. Chronic obstructive pulmonary disease and intestinal ischemia were reported in different patients and were deemed serious AEs. No serious AE was associated with CDZ exposure [17]. No completed study to date has enrolled over 84 patients. Further investigations are required to determine the true incidence and extent of AEs.

2.3.3. Pharmacokinetics

Low CDZ plasma concentrations have been noted in each of the trials, indicating minimal absorption. In the phase I trial with healthy volunteers, the mean plasma concentrations (C_{max}) ranged from 0.91 to 2.28 ng/mL on day 1 and 1.82–3.28 ng/mL on day 10 [14]. The time to maximum plasma concentration (T_{max}) ranged from 1 to 4 h throughout the study. Following a single oral dose, 81–86.1% of CDZ was recovered unchanged in the feces. In the MAD arm of the study, average fecal concentrations were 2.4 mg/g (300 mg), 9.4 mg/g (1000 mg), and 27.7 mg/g (3000 mg). Urinary excretion was noted to range from 0.001% to 0.012% of the administered drug. In severe CDI, the mean C_{max} was 2.64 ng/mL [16]. In the phase II trial, the C_{max} of CDZ was 18.9 ng/mL in the 1000 mg BID arm [17]. Median C_{max} was 1.22, 1.62, and 2.07 ng/mL in the 250, 500, and 1000 mg BID arms, respectively. No further pharmacokinetic parameters were disclosed for the study.

2.3.4. Pharmacodynamics

Limited clinical data have been disclosed on the effects that CDZ has on GI microflora. No major effects on the GI microbiome were reported in patients receiving up to 1000 mg BID during phase II trials [6].

3. LFF571

3.1. Novartis Pharmaceuticals

LFF571 (Figure 3) is a novel cyclic peptide in early clinical stage development by Novartis (Basel, Switzerland). As part of an emerging class of thiopeptide antibiotics, LFF571 is a semisynthetic derivative of a natural metabolite (GE2270 A) from the bacterium *Planobispora rosea* [19]. Mechanistically, LFF571 inhibits elongation factor Tu (EF-Tu), a protein required for peptide synthesis [20]. Cross-resistance is thought to be negligible since there are no antibiotics in use that share this unique mechanism of action. LFF571 exhibits potent *in vitro* activity against *C. difficile*, other Gram-positive anaerobes, and select Gram-positive aerobes [21]. As of June 2016, Novartis completed an exploratory phase II trial (NCT01232595) of LFF571 in North America to compare safety and efficacy with VAN [22].

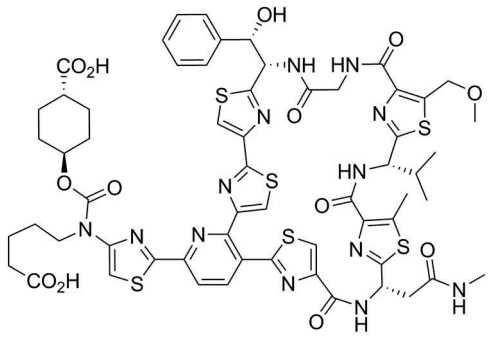

Figure 3. Chemical structure of LFF571 (MW 1366.6).

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3.2. Preclinical development

3.2.1. Microbiology

The activity spectrum of LFF571 includes Gram-positive anaerobes and aerobes (Table 3). Clinically relevant *S. aureus* and *Enterococcus* strains were sensitive with an MIC range of 0.015–0.25 µg/mL [21]. When tested against 50 strains of *C. difficile*, LFF571 had an MIC_{90} of 0.25 µg/mL compared to 0.5 µg/ml for FID and 2 µg/mL for both MET and VAN. Similar patterns of susceptibility were detected in clinical isolates of *C. difficile* from Europe and for ribotype 027 strains [23]. The lack of significant activity against Gram-negative anaerobes suggests that LFF571 may have an attenuated impact on the normal flora of the GI tract.

Resistance development is a potential problem for antibiotics that inhibit protein synthesis (e.g. macrolides and clindamycin). To probe potential mechanisms of resistance, *C. difficile* was cultured with increasing LFF571 concentrations to select for resistance [24]. The resistance frequency was found to be in the range of $<4.5 \times 10^{-11}$ to 1.2×10^{-9} and the MICs of the resistant isolates ≥128 µg/mL. A one to two amino substitution in the EF-Tu protein was attributed to the resistance development. In a subsequent study, the same research group was unable to select for resistance in serially passed *C. difficile* cultures [24]. Because serial passage testing uses repeated exposures at subinhibitory concentrations, it is hypothesized that *C. difficile* would not have the capability to quickly develop resistance to LFF571 *in vivo*. Moreover, subinhibitory levels of LFF571 showed reduced toxin production in cultured *C. difficile* [25]. Similar *in vitro* effects were observed with FID, while VAN and MET increased toxin production in some strains; however, these effects have not been studied *in vivo* for LFF571.

3.2.2. Animal studies

LFF571 (MW 1366) is a water-soluble peptide (12 mg/mL, pH 7.4) with low oral bioavailability [26]. Unlike most other peptide antibiotics, LFF571 operates intracellularly on the EF-Tu protein. For this reason, the optimal balance between the pharmacodynamic and pharmacokinetic properties posed a challenge to developers. The efficacy of LFF571 has been tested against VAN in a CDI hamster model [27]. LFF571 dosed at 5 mg/kg was found to significantly decrease the hazard of death associated with VAN dosed at 20 mg/kg (69% decrease; $p = .0022$). Of the pooled treatment groups, 10 of 54 animal deaths occurred in the LFF571 group compared to 28 of 56 deaths in the VAN group. The study further found a significant reduction in recurrence hazard with LFF571 (95% decrease; $p = .0024$); however, it was noted that VAN may have been underdosed.

Table 3. Comparison of antibacterial activity of LFF571 with standard drugs [21,23].

	MIC_{90} (µg/mL)			
Species (strains)	MET	VAN	FID	LFF571
Clostridium difficile (50)	2	2	0.5	0.25
C. difficile ribotype 027 (18)	1	0.5	0.5	0.25
Clostridium innocuum (10)	1	16	>32	0.25
Clostridium perfringens (20)	2	1	≤0.015	0.03
Bacteroides fragilis (21)	1	32	>32	8
Bifidobacterium spp. (22)	>64	1	0.125	>32
Eubacterium limosum (20)	1	2	>32	0.25
Fusobacterium nucleatum (22)	0.5	>32	>32	32
Lactobacillus spp. (24)	>64	>64	32	>32
Prevotella bivia (20)	4	>64	>32	>32
Staphylococcus aureus (20)	–	1	8	0.125
Streptococcus pyogenes (21)	–	≤0.25	8	2
Enterococcus faecalis (22)	–	>16	4	0.03
Enterococcus faecium (20)	–	>16	4	0.06
Peptostreptococcus spp. (20)	1	0.5	≤0.015	0.06

MET: metronidazole; VAN: vancomycin; FID: fidaxomicin.

3.3. Clinical trials

A randomized, double-blind, placebo-controlled, SAD and MAD phase I trial was conducted to determine the safety, tolerability, and pharmacokinetics of LFF571 in healthy volunteers [26]. Subjects were enrolled in either the MAD or the SAD arm. Subjects in the single-dose cohort were given LFF571 25, 100, 400, and 1000 mg with a high-fat meal, while the subjects in the MAD arm received 25, 100, or 200 mg QID, without regard to food, for 10 days. The results were determined to support further development of LFF571 for the treatment of CDI. A randomized, evaluator-blind, active-controlled, parallel-group phase II trial (NCT01232595) evaluated the safety and tolerability of LFF571 and VAN in moderate CDI patients [22,28]. Subjects were randomized to receive either 200 mg of LFF571 or 125 mg of VAN QID for 10 days. The primary end point was the proportion of clinical cures at the end of the study period.

3.3.1. Dosage and administration

Three ascending doses of LFF571 were tested for safety and tolerability [26]. All doses were well tolerated, and systemic absorption did show linear increase with increasing doses. Serum levels were higher when LFF571 was taken under fasted conditions in healthy volunteers, but levels were still minimal and most could not be quantified. The maximum tested dose in the multiple-dose arm of the phase I trial was 200 mg every 6 h, which was also the dosing regimen used for the phase II trial. Although there have been no phase III trials to further investigate this dose, it seems likely that the standard dose will be 200 mg every 6 h, given without regard to meals.

3.3.2. Safety and tolerability

In the phase I trial, single 25, 100, 400, and 1000 mg doses of LFF571 were administered to healthy subjects, in addition to repeated doses of 25, 100, and 200 mg given QID for 10 days [26]. LFF571 was found to be safe and well tolerated across all doses, and AEs did not seem to increase with higher doses. The most frequent side effects were GI related. Diarrhea was present in all groups with no statistically significant difference noted. No subjects withdrew from treatment due to GI events. The AE profile in subjects with moderate CDI given 200 mg QID in the phase II trial was similar to that of the healthy volunteers [22].

While the incidence of AEs reported for the LFF571 arm was higher than that for the VAN arm of the phase II trial, more of the AEs in the VAN arm were deemed to be due to the treatment [22]. AEs occurring with greater frequency in the LFF571 arm included anxiety and abdominal pain. The VAN group had a higher incidence of back pain, upper respiratory infections, and hematochezia. The incidence of serious AEs

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was similar between the groups and included *C. difficile* colitis and urinary tract infection. *C. difficile* colitis occurred in two patients in each of the treatment arms, but remaining serious AEs were reported in only one patient each. The only events that were deemed possibly related to LFF571 were leukocytosis and *C. difficile* colitis [22]. Both trials had a small enrollment, and the true incidence of AEs related to LFF571 requires further investigation.

3.3.3. Pharmacokinetics

Both trials noted that many pharmacokinetic parameters were unable to be calculated due to low serum concentrations of LFF571. Single doses up to 1000 mg and multiple doses of 200 mg every 6 h resulted in limited systemic exposure [26]. In the phase II trial, the highest observed value was 41.7 ng/mL [28]. Higher levels in subjects with moderate CDI suggest increased absorption when there is intestinal inflammation. Maximum concentrations are attained within 3 h post-dose. Mean C_{max} ranged from 4.17 to 7.22 ng/mL. Area under the curve up to the last measurable concentration (AUC_{last}) varied greatly on observed days with a mean of 11.6, 23.5, and 20.7 h·ng/mL on days 1, 3, and 10, respectively. Mean fecal levels of LFF571 in the phase II trial were 3950 µg/mL. The relatively high fecal concentration of LFF571 combined with the low serum concentration suggests that it is likely to remain in the GI tract and have limited systemic absorption [28]. First-pass hepatobiliary metabolism has not been demonstrated in animal or human models. Urine drug levels were not tested for LFF571, but given the limited systemic absorption, there will likely be no significant renal excretion.

3.3.4. Pharmacodynamics

To our knowledge, no clinical trial data on the effects of LFF571 treatment on the human GI microbiota in healthy or CDI patients have been reported prior to June 2016.

4. Ridinilazole (SMT19969)

4.1. Summit Therapeutics plc

Ridinilazole (RID) (SMT19969) (Figure 4) is a synthetic bis-(4-pyridyl)-benzimidazole in late-stage development by Summit Therapeutics (Abingdon, United Kingdom) [29]. The compound belongs to a novel class of heterocyclic antibacterials, which were originally discovered by university researchers who found that they possessed narrow-spectrum activities against Gram-positive pathogens [30]. Through a subsequent academic–industrial partnership, Summit Therapeutics developed the compounds for the treatment of CDI with minimal impact on the GI microflora. Lead optimization studies identified RID with the optimal combination of physicochemical properties for a minimally absorbed oral drug possessing highly selective bactericidal activity against *C. difficile*. In November 2015, Summit Therapeutics announced plans to continue the clinical trials of RID after achieving statistical superiority over VAN on sustained clinical response [31]. To expedite approval, RID has been granted QIDP designation and Fast Track status.

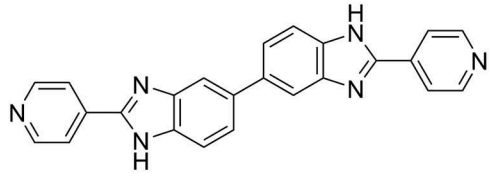

Figure 4. Chemical structure of ridinilazole (MW 388.4).

4.2. Preclinical development

4.2.1. Microbiology

RID functions as an inhibitor of DNA synthesis in bacteria through one or more mechanisms [30]. In a recent study, RID was found to induce cell elongation while inhibiting sporulation, in contrast to VAN and MET [32]. RID exhibits minimal activity on cultured gut microflora bacteria with >1000 selectivity against important GI species [33–37]. Susceptible species with an MIC_{90} of ≤1 µg/mL include *C. difficile*, *Clostridium innocuum, Parvimonas micra*, and *Porphyromonas* spp. (Table 4). Facultative anaerobes with low susceptibility levels include lactobacilli, staphylococci, streptococci, and enterococci. Of particular note, colonic anaerobes *B. fragilis, Prevotella* spp., and bifidobacteria, which are thought to play a critical role in preventing colonization and overgrowth of *C. difficile*, were resistant to RID at high MIC concentrations. Overall, RID exhibited comparable potency and activity spectrum to FID with the exception that Gram-positive cocci and *Bifidobacterium* spp. were generally more sensitive to the latter.

Against a multiple polymerase chain reaction-ribotype panel of *C. difficile* including 027, RID demonstrated comparable activity to FID [30]. Time-kill studies further revealed that the killing effects of RID against cultured *C. difficile* initiated within 8 h and were independent of drug concentration [36]. A post-antibiotic effect of 4–20 h at 10x MIC was observed. Drug efficacy using an *in vitro* gut model revealed that RID was 7 and 17 times more effective than MET and VAN, respectively, in eradicating *C. difficile* ribotype 027 when using similar clinical regimens [37]. During the 7-day installation period,

Table 4. Comparison of antibacterial activity of ridinilazole with standard drugs [30,34].

	MIC_{90} (µg/mL)			
Species (strains)	MET	VAN	FID	RID
Clostridium difficile (50)	2	4	0.5	0.25
C. difficile ribotype 027 (11)	8	4	0.5	0.25
Clostridium innocuum (10)	2	16	256	1
Clostridium perfringens (11)	4	1	0.06	>512
Bacteroides fragilis (20)	2	64	>512	>512
Bifidobacterium spp. (14)	128	1	0.125	>512
Eggerthella lenta (20)	0.5	2	≥0.03	>512
Fusobacterium nucleatum (10)	0.25	512	>512	64
Lactobacillus spp. (20)	>512	>512	>512	>512
Prevotella spp. (23)	1	>512	16	>512
Staphylococcus aureus (10)	>512	1	16	>512
Streptococcus spp. (10)	>512	1	32	>512
Enterococcus faecalis (10)	>512	4	8	>512
Enterococcus faecium (10)	>512	256	8	128
Peptostreptococcus anaerobius (20)	1	0.5	≥0.03	64

MET: metronidazole; VAN: vancomycin; FID: fidaxomicin; RID: ridinilazole.

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immediate declines in vegetative forms and cytotoxin titer were noted for RID. Reduction in toxin A/B levels of 80–90% was similarly observed for RID-treated cultures of *C. difficile* ribotype 027 [32]. The study concluded that anaerobic and facultative anaerobic microflora remained mostly intact with RID treatment. Conversely, MET and VAN can shift the gut population from anaerobic to facultative anaerobic species-dominated microflora facilitating VRE overgrowth.

4.2.2. Animal studies

Preclinical pharmacokinetic studies utilized oral dosing of male Golden Syrian hamsters with 0.5% aqueous methylcellulose solution of RID at 20 mg/kg [38]. Plasma drug concentrations at days 1 and 5 were below the minimum limits of detection (25 ng/mL) indicating minimal systemic exposure. Fecal analysis at post-dose time points 1–6 h showed drug concentrations ranged from 96 to 172 µg/mL, a level >100-fold higher than the MIC_{90} for *C. difficile*. Similar concentrations were observed on day 5 (109–146 µg/mL), and it was concluded that no significant drug accumulation occurs for RID.

RID was evaluated in a comparative survival rate study with VAN using a CDI hamster model [38]. In the nonepidemic VA11 strain infection model, animals dosed at 10–50 mg/kg had a survival of 80–95% compared to 100% receiving 20 mg/kg VAN. Animals infected with epidemic VA5 strain were fully protected by 50 mg/kg RID up to 1-week posttreatment. RID was further compared with VAN and FID in the same model using ribotype 027 and 012 variants [39]. During the 5-day dosing period, each drug provided protection with 100% survival. High survival rates were observed for up to 28 days following RID treatment; however, spores were detected in the feces at days 19 and 28 postinfection.

4.3. Clinical trials

From 2014 to 2015, a randomized phase I study (ISRCTN10858225) was performed to assess the safety, tolerability, pharmacokinetics, and impact on the gut microbiota for RID [29,40]. The double-blinded, placebo-controlled study evaluated single and multiple oral dosing in 56 healthy males. The fasting and fed effects of 2–2000-mg dose regimens were examined in the study [40]. After meeting outcome measures, a phase II proof-of-concept clinical trial (CoDIFy, NCT02092935) was conducted in the US and Canada. The double-blind, randomized, multicenter study enrolled 100 CDI patients to receive either RID 200 mg BID or VAN 125 mg QID. In late 2015, Summit Therapeutics announced that RID achieved statistical superiority over VAN in the trials, with sustained clinical response rates of 66.7% and 42.4%, respectively [31]. In the same trial, RID demonstrated a higher clinical cure rate (77.8% vs. 69.7%) and lower recurrence rate (14.3% vs. 34.8%) in the modified intent-to-treat population for CDI patients with free toxin present in feces [41].

4.3.1. Dosage and administration

During the phase I study, RID was provided as an oral suspension for reconstitution in 30-mL bottles. Dosages used for extended days included 200 and 500 mg BID. Both were provided within an hour following a light meal for 10 days [40]. In the CoDIFy phase II study, the dosage used was 200 mg BID for 10 days (dosage form not specified) [41].

4.3.2. Safety and tolerability

During the phase I clinical trial, RID did not show any treatment- or dose-related trend in the incidence of AEs. The majority of AEs were classified as GI disorders, resolved without treatment, and were mild in severity. The only serious AE that occurred was acute appendicitis, which was considered probably unrelated to RID. Other AEs that occurred in patients with large single doses (2000 mg) of RID include abdominal distention, duodeno-gastric reflux, and feelings of excessive warmth [40]. Patients who received 10 days of treatment experienced diarrhea, abdominal pain, and paresthesia. During the phase II clinical trial comparing efficacy with VAN, no safety signals were identified with RID treatment [41]. Both treatment arms had similar rates of AEs (80%) and serious AEs (17%).

4.3.3. Pharmacokinetics

Phase I trials discerned that >97% of RID remains unabsorbed and passes unchanged via fecal matter [40]. Single doses up to 2000 mg result in a low C_{max} (0.102–0.296 ng/mL). After repeated administration (200–500 mg) in the fed state for 10 days, plasma concentrations remained minimal (0.105–0.305 ng/mL). C_{max} was consistently achieved 4 h post-RID administration.

4.3.4. Pharmacodynamics

The effects of RID on the GI microflora in healthy patients have been described for the phase I trial [40]. Fecal samples collected up to day 9 during treatment with 200 or 500 mg BID were evaluated. With the exception of clostridia, minimal alteration in microflora composition was observed. A slight elevation in bacteroides (0.5–2.0 log), total aerobes (2.0 log), and lactose-fermenting Enterobacteriaceae (LFE) (2.0 log) populations were noted for the 200-mg BID treatment group. Lactobacilli counts (1–4 log) decreased moderately, while total anaerobes and bifidobacteria levels generally remained constant. In the 500-mg BID group, RID reduced the clostridia population below the limit of detection, and bacteroides counts decreased slightly with larger RID doses.

5. Surotomycin (CB-183,315)

5.1. Cubist Pharmaceuticals/Merck & Co

Surotomycin (SUR) (CB-183,315) is a semisynthetic lipopeptide in late-stage clinical development from Cubist Pharmaceuticals (Lexington, MA, USA), a subsidiary of Merck & Co. The narrow-spectrum cyclic peptide is obtained from the natural product daptomycin (DAP) (Cubicin™) by enzymatic cleavage of the decanoyl side chain and installation of the (*E*)-3-(4-pentylphenyl)-but-2-enoyl residue in that position (Figure 5) [42]. The two compounds share the same mechanism of action as calcium-dependent cell membrane-depolarizing agents [43]. SUR is a water-soluble, 13-amino acid peptide with low oral bioavailability (<1%) and is excreted in feces, resulting in high GI concentrations. As of January 2016, Cubist concluded phase III trials to compare the efficacy of SUR to VAN as a clinical cure for CDI. SUR has received both the Fast Track and QIDP regulatory designation by the FDA.

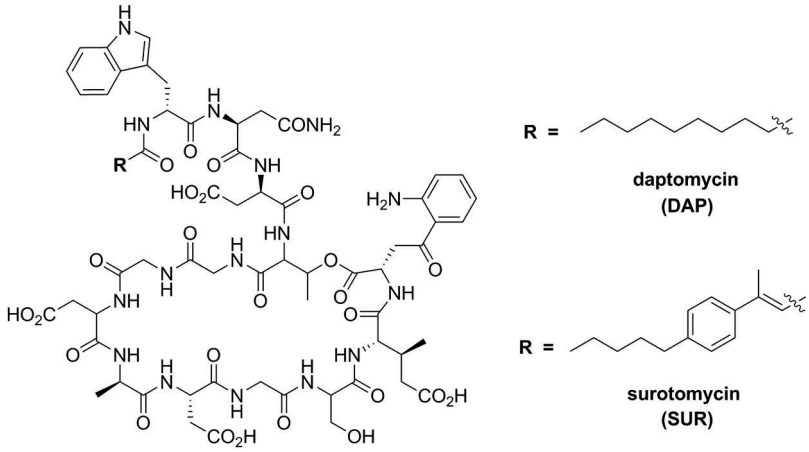

Figure 5. Chemical structure of surotomycin (MW 1680.7), a daptomycin-derived lipopeptide.

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5.2. Preclinical development

5.2.1. Microbiology

Like DAP, SUR does not penetrate the outer membrane of Gram-negative bacteria and can only elicit bactericidal activity against Gram-positive species. SUR demonstrated 4x the activity of DAP against 30 strains of *C. difficile* (MIC_{90} 0.5 µg/mL) that included eleven 027 ribotype variants [42]. In a study with 55 clinical isolates, SUR demonstrated an MIC_{50} of ≤0.125 µg/mL and an MIC_{90} of 0.25 µg/mL against the panel that included MET-, VAN-, and FQ-resistant *C. difficile* strains (Table 5) [44]. Reproducibility in both liquid and agar culture was confirmed in a study with 103 clinical isolates [45]. Other clinically relevant bacteria with susceptibility to SUR are MRSA and VRE with MIC ranges of 1 to 2 µg/mL. Notable gut microflora activity was limited to Gram-positive bacteria including *Bifidobacterium, Peptostreptococcus*, and *Lactobacillus* spp.

Further preclinical studies assessed SUR in an *in vitro* gut model of clindamycin-induced CDI. SUR and VAN exhibited comparable efficacy to resolve CDI but had different effects on the GI microbiota [46]. SUR was more damaging to clostridia and lactobacilli populations, while *B. fragilis* levels were unaffected in contrast to high-dose VAN. Low frequency of resistance is noted for SUR, although bioinformatics studies on serially passed *C. difficile* with reduced susceptibility revealed that resistance might be evoked by a mutation in cardiolipin synthetase [47]. Additional *in vitro* studies resolved that SUR demonstrates rapid bactericidal action and, unlike MET and VAN, maintains activity against stationary-phase *C. difficile* [48,49]. It is speculated that SUR may be advantageous over existing therapies due to the bactericidal effects it elicits on stationary-phase cells when spore and exotoxin production levels are highest.

Table 5. Comparison of antibacterial activity of surotomycin with standard drugs [44].

	MIC_{90} (µg/mL)		
Species (strains)	MET	VAN	SUR
Clostridium difficile (55)	16	4	0.25
Clostridium perfringens (20)	–	2	0.5
Clostridium spp. (33)	8	4	2
Bacteroides fragilis (21)	2	128	>8192
Bifidobacterium spp. (14)	–	1	2
Eubacterium limosum (13)	–	2	0.5
Fusobacterium necrophorum (20)	0.5	>8192	8192
Lactobacillus spp. (37)	–	2 to >32	2 to 4
Prevotella spp. (20)	4	256	>8192
Staphylococcus aureus – MSSA (12)	–	2	0.5
S. aureus – MRSA (12)	–	2	1
Enterococcus spp. (60)	–	>64	2
Enterococcus – VRE (21)	–	>64	2
Peptostreptococcus (45)	–	1	0.5
Anaerobic Gram-positive cocci (49)	–	0.5	0.5

MET: metronidazole; VAN: vancomycin; SUR: surotomycin; MSSA: methicillin-susceptible Staphylococcus aureus; MRSA: methicillin-resistant *Staphylococcus aureus*; VRE: vancomycin-resistant enterococcus.

5.2.2. Animal studies

Survival rate studies were performed using a CDI hamster model infected with toxin B-producing *C. difficile* [50]. Animals dosed orally with 2–25 mg/kg BID × 5 days were consistently protected from the initial infection by SUR and VAN. During the CDI recurrence phase from days 6 to 35 posttreatment, the SUR- and VAN-treated groups exhibited similar dose- and time-dependent survival.

5.3. Clinical trials

Cubist Pharmaceuticals completed phase I–III trials on SUR. Single- and multiple-dose studies during phase I trials assessed safety, tolerability, pharmacokinetics, and effect on the GI microflora in healthy volunteers. After meeting the outcome measures, a randomized, double-blind, multicenter phase II trial (NCT01085591) compared 125 and 250 mg BID regimens to VAN 125 mg QID in 209 subjects [8,51]. The CDI response rates (%) for the three respective cohorts were compared by cure rate at the termination of therapy (92.4, 86.6, and 89.4). Recurrence rates (27.9, 17.2, and 35.6) and sustained cure rates (66.7, 70.1, and 56.1) were evaluated after 4 weeks. The study concluded that SUR 250 mg BID met the outcome measures and would advance to phase III trials. In March 2015, a phase III trial to compare SUR 250 mg BID to VAN 125 mg QID was completed. The randomized, double-blind study (NCT01597505) conducted with 608 adult subjects has not reported results as of June 2016 [8].

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5.3.1. Dosage and administration

Oral tablets of SUR were provided to healthy patients in phase I studies for once- or twice-daily dosing [52,53]. Doses ranged from 0.25 to 4 g in the SAD study and 0.25 to 1 g BID in the MAD study. The phase II trial utilized 125 and 250 mg BID oral dosing of SUR for 10 days [8,51].

5.3.2. Safety and tolerability

Like DAP, oral SUR is not well absorbed, and systemic effects are expected to be minimal. During the phase I trial, AEs were mild to moderate with single oral doses up to 4 g [52,53]. Each event was considered unlikely/not related to SUR exposure. During the phase II trial (NCT01085591), serious AEs were limited to 8.7% for patients receiving SUR compared to 15.7% for oral VAN [8,51]. The percentages for other AEs for SUR and VAN cohorts were 62.3% and 58.6%, respectively. The most frequent AEs were headache (12%) and nausea (12%).

5.3.3. Pharmacokinetics

During a phase I SAD study, healthy volunteers received doses of 0.5, 1, 2, and 4 g of SUR [52]. The median C_{max} was 10.5 and 86.7 ng/mL for the 0.5 and 4 g single-dose cohorts, respectively. The median elimination half-life ranged from 14.8 to 21.1 h and fraction excreted in urine was <0.01%. In the MAD study, three cohorts of 250, 500, and 1000 mg SUR BID for 1–14 days were examined. The median C_{max} ranged from 6.8 to 21.0 ng/mL (day 1) to 25.5–93.5 ng/mL (day 14). The mean fecal concentration of SUR in the 1000-mg BID cohort was 6.39 mg/g on day 5.

5.3.4. Pharmacodynamics

The effects of SUR on the GI microflora in healthy patients have been described in a phase I trial [53]. Fecal samples in patients treated with SUR 250–1000 mg BID were collected at baseline (day 0–1) and up to day 13–15. The study established that SUR had minimal disruptive effects on Gram-negative anaerobes compared to VAN. Notwithstanding, clostridia, streptococci, and enterococci had lower postexposure counts. Lactobacilli and bifidobacteria were also reduced to a lower extent, while *B. fragilis, Prevotella* spp., and enterobacteria (*E. coli, Klebsiella* spp., and *Enterobacter* spp.) were generally unaffected. Overall, SUR caused a 1 to 2 $\log_{10}$ colony-forming unit (CFU)/g reduction, having the greatest impact on the Gram-positive species population.

6. Expert commentary

CDI is among the most challenging infections to overcome due to the limited susceptibility of *C. difficile* to common antibiotics and its ability to outlast treatment through sporulation. Current therapies have various drawbacks, and new drugs are sought to improve sustained response and decrease relapse rates with minimal impact on the GI microbiome. The experimental treatments described in this review are most advanced in clinical development for CDI although LFF571 appears to be no longer in development. Comparison of data between the medications suggests that RID may have the least disruptive effects on the GI microbiota and lowest risk for VRE overgrowth due to the limited susceptibility of important colonic anaerobes *B. fragilis, Prevotella* spp., and bifidobacteria (Table 6) [30,34,40]. Both RID and CDZ additionally show evidence of reducing sporulation in preclinical studies [13,32] and each outperformed VAN in phase II trials for clinical cure and sustained cure rates [17,41].

Notwithstanding, RID, CDZ, and SUR have shown the ability to lower recurrence rates compared to VAN [8,17,41]. Unlike MET, but similar to VAN, all of these agents appear to remain confined in the GI tract which should limit AEs. Each antibiotic displayed a low percentage of serious AEs in clinical trials that was attributed to drug exposure [14–17,40,51]. Among the four therapies reviewed,

Table 6. Comparison of preclinical and clinical trial data [8, 7, 14, 16–18, 21, 22, 26, 34, 40, 41, 44, 52].

	CDZ	LFF571	RID	SUR
Preclinical	MIC range (μg/mL)			
C. difficile	0.06–0.5	0.125–0.5	0.125–0.5	≤0.125–1
B. fragilis	2–8	>32	512–>512	>16
Prevotella spp.	–	>32	32–>512	≥8192
Bifidobacterium spp.	0.5	>32	16–>512	0.06–2
Lactobacillus spp.	0.5	0.06–>32	0.06–>512	0.125–16
Enterococcus spp.	0.25–2	≤0.015–0.06	64–>512	≤0.125–2
Clinical trials	250 mg BID	200 mg QID	200 mg BID	250 mg BID
Median plasma concentration (ng/mL)	0.1–0.9	3–4	0.1	–
Fecal drug concentration (μg/mg)	101–2710	107–12,900	847–2390	–
Clinical cure rate (%, VAN)	77 (68)	91 (78)	78 (70)	87 (89)
Recurrence rate (%, VAN)	18 (50)	19 (25)	14 (35)	17 (36)
Sustained cure rate (%, VAN)	60 (33)	57 (65)	67 (42)	70 (56)
Adverse events (%, VAN)	30 (46)	76 (69)	80 (80)	62 (59)
Adverse events (≥5%, %)	Headache (10)	Anxiety (11)	–	Headache (12)
	Dizziness (5)	Nausea (9)		Nausea (12)
	Dyspepsia (5)	Abdominal pain (9)		Fatigue (7)
		Constipation (7)		Abdominal pain (6)
				Urinary infection (6)

CDZ: cadazolid; RID: ridinilazole; SUR: surotomycin; VAN: vancomycin; MIC: minimum inhibitory concentration; BID: two times a day; QID: four times a day.

CDZ appears to exhibit the lowest frequency of AEs. Of note, none of these agents have been formally compared to oral FID in humans; however, Summit Therapeutics is currently recruiting 30 patients for a phase II trial for comparison of RID with FID (NCT02784002). Clinical data suggest that FID is superior to VAN in both treating and preventing recurrent CDI [54]. The data presently available has indicated that each of these experimental drugs may also provide high cure rates of CDI without recurrence.

7. Five-year view

The four antibiotics reviewed appear to have distinct advantages over current therapies with respect to their pharmacodynamic and safety profiles. Based on the information available, CDZ and RID are progressing at rate that will enable them to be approved by 2021. Actelion Pharmaceuticals is currently recruiting CDI patients for a phase III multicenter, randomized, double-blind study (NCT01983683) with an expected completion date of February 2017 [8]. Moreover, we anticipate that RID will enter phase III trials based on the positive phase II results [31]. If the outcome measures are met and the medications receive approval, the cost of the treatments to patients will have a significant influence on their ultimate use and place in CDI therapy.

Key issues

- There is high recurrence rate in patients treated for gastrointestinal infections due to *Clostridium difficile*, a spore-forming multidrug-resistant anaerobe.
- Cadazolid, LFF571, ridinilazole, and surotomycin have emerged as potential therapies with improved clinical and sustained cure rates to existing treatments.
- Each of these investigational drugs exhibit low absorption (< 1%) permitting delivery of supratherapeutic doses (≥ 100 x MIC) to the infection site. In turn, there is minimal systemic exposure to provoke drug-related adverse effects.
- Among the drugs reviewed, ridinilazole appears to have least disruptive effects on gastrointestinal microbiome bacteria including anaerobes and *Enterococcus*.
- Phase II trials with vancomycin as comparator indicate that cadazolid, ridinilazole, and surotomycin have higher sustained cure rates at 30 days post-treatment. This endpoint was not achieved for LFF571, which appears to no longer be in development.
- Cadazolid and ridinilazole have shown evidence to reduce sporulation rates in *Clostridium difficile*.
- Narrow-spectrum agents that can eliminate sporulation and toxin-production while eradicating both dividing and non-dividing cells are expected to be the most effective sustained cures for *C. difficile* infections.

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