ABSTRACT

In spite of increased awareness and the efforts taken to optimize Clostridium difficile infection (CDI) management, with the limited number of currently available antibiotics for C. difficile the halt of this increasing epidemic remains out of reach. There are, however, close to 80 alternative treatment methods with controversial anti-clostridial efficacy or in experimental phase today. Indeed, some of these therapies are expected to become acknowledged members of the recommended anti-CDI arsenal within the next few years. None of these alternative treatment methods can respond in itself to all the major challenges of CDI management, which are primary prophylaxis in the susceptible population, clinical cure of severe cases, prevention of recurrences, and forestallment of asymptomatic C. difficile carriage and in-hospital spread. Yet, the greater the variety of treatment choices on hand, the better combination strategies can be developed to reach these goals in the future. The aim of this article is to provide a comprehensive summary of these experimental and currently off-label therapeutic options.

Keywords: Clinical development pipeline; Clostridium difficile infection (CDI); Controversial therapies; Experimental therapies

INTRODUCTION

Clostridium difficile infection (CDI) has been increasingly recognized in recent years as an entity of primary importance that requires prompt diagnosis and efficient treatment to prevent a severe and complicated disease course, in-hospital spread and recurrences. However, due to the spore-forming ability of
C. difficile, the impaired host intestinal microbiota and the altered immunity of CDI patients, current treatment strategies often have suboptimal results with regard to clinical cure and relapse prevention alike. In fact, present therapeutic options leave ample room for improvement in terms of clinical outcome, as the average cure rates achieved by the three main antibiotics currently recommended for CDI treatment (metronidazole, vancomycin and fidaxomicin [1, 2]) do not exceed 80–90% [3]. Although fidaxomicin boasts significantly lower relapse rates than the other two alternatives, sustained cure (that is cure without recurrence) may be as low as 75% among patients treated with this best available option [4, 5].

The urgent need for more efficient tools to fight CDI makes it unsurprising that today there are an impressive number of novel antibiotics and other therapies at different stages of development, some of them already being tested in phase III randomized controlled trials (RCTs). At the same time, some long-known drugs that are currently not recommended for CDI have been revisited lately to check whether there is potential room for them in the arsenal of anti-CDI therapeutics. The aim of this review is to summarize novel, neglected and controversial CDI treatment options, some of which may become part of everyday practice in the near future.

There are various criteria according to which one may classify these therapies (mode of administration, predominantly prophylactic vs. therapeutic agents, natural vs. synthetic products, etc.). In this article we resume them according to the major therapeutic effect that is sought by their application (Fig. 1). Accordingly, the majority of these products can fit in one of the following categories: (1) antibiotics and non-antibiotic agents with bacteriostatic/bactericidal effect against C. difficile; (2) toxin-neutralizing agents; (3) therapies that boost host immune defense against CDI; (4) treatments that modulate the intestinal environment to make it less favorable for C. difficile colonization; (5) anti-inflammatory substances that prevent or reduce enterocyte damage caused by C. difficile toxins. Table 1 summarizes all therapies discussed in the following, along with their current phase of development.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

ANTIBIOTICS AND NON-ANTIBIOTIC ANTICLOSTRIDIAL AGENTS

Similarly to the three main currently recommended anti-clostridial drugs, the majority of off-label and experimental therapeutic options seek to have a direct effect on the causative microorganism. These antibiotics and non-antibiotic agents target certain molecular components of C. difficile with the aim of eliminating the bacteria in an already established infection. They are presented in the following, grouped according to their main mechanism of action.

Inhibitors of Transcription and DNA Synthesis

**Rifamycins**

Rifaximin is a semisynthetic, nonabsorbable derivative of rifamycin that inhibits bacterial
RNA synthesis, primarily used in the treatment of traveler’s diarrhea and hepatic encephalopathy. It is considered to have very little and rather beneficial effect on the normal intestinal microbiota [6, 7], though its complete innocuousness in patients receiving long-term rifaximin treatment is questionable [8]. It was equally efficient as vancomycin in a hamster model of CDI, and—depending on the C. difficile strain used—similar or lower recurrence rates were observed after rifaximin withdrawal as compared to the vancomycin group [9]. There have been various retrospective and prospective clinical case series demonstrating its efficacy in first CDI episodes, as well as in recurrent and refractory CDI [10–13], but rifaximin failed to unmistakably demonstrate non-inferiority to vancomycin in a RCT [14]. Its somewhat higher resistance rates as compared to vancomycin and metronidazole—especially

![Image](image_url)
| Drug type | Mode of action | Target | Compound | Investigation phase | References |
|-----------|----------------|--------|----------|---------------------|------------|
| Antibiotics and non-antibiotic anti-clostridial therapies | Inhibition of replication and transcription | DNA gyrase/topoisomerase | Kibdelomycin | In vivo preclinical | [25] |
| | RNA-polymerase | | OPS-2071 | Phase II ongoing | [30] |
| | | | Rifaximin | Phase IV ongoing* | [23] |
| | | | Rifaxalazil | In vivo preclinical* | [24] |
| | Minor groove of DNA | | MGB-BP-3 | Phase I completed | [27] |
| | Inhibition of protein synthesis | 30 s ribosome subunit | Tigecycline | In vivo preclinical* | [35] |
| | | 50 s ribosome subunit | Omadacyclin | In vivo preclinical* | [42] |
| | | | RBx 11760 | In vivo preclinical | [56] |
| | | | RBx 14255 | In vivo preclinical | [57] |
| | | Elongation factor G | Fusidic acid | Phase III completed | [45] |
| | | Elongation factor tu | LFF571 | Phase II completed | [51] |
| | | Methionyl-tRNA synthetase mRNA | CRS 3123 | Phase I ongoing | [55] |
| | | Bolasome-antisense gapmer nanocomplexes | In vitro preclinical | | [59] |
| | Inhibition of metabolic pathways | Pyruvate:ferredoxin oxidoreductase | Nitazoxamide | Phase III completed | [65] |
| | | | Amixicile | In vivo preclinical | [68] |
| | Inhibition of cell-wall formation | D-Ala-D-Ala terminal of peptidoglican | Teicoplanin | Phase III completed* | [44, 73] |
| | | | Ramoplanin | Phase III completed | [80] |
| | | | Oritavancin | In vivo preclinical* | [84] |
| Drug type                          | Compound          | Target                              | Mode of action                                      | Investigation phase | References |
|-----------------------------------|-------------------|-------------------------------------|-----------------------------------------------------|---------------------|------------|
| N-substituted methylvancomycin    | Actagardine       | Cell membrane and cell wall structures | In vitro preclinical                                |                    | [88]       |
| N-substituted methylvancomycin    | V15F              | Cell membrane                        | In vitro preclinical                                | Phase I completed   | [89]       |
| N-substituted methylvancomycin    | NVB302            | Cell membrane                        | In vivo preclinical                                 | Phase III completed | [90]       |
| N-substituted methylvancomycin    | SQ641             | Translocase I                        | In vitro preclinical                                |                    | [91]       |
| Lipid II                          | Surotomycin       | Cell membrane and cell wall          | In vivo preclinical                                 | Phase III completed | [92]       |
| Lipid II                          | Thuricin CD       | Cell membrane                        | In vitro preclinical                                |                    | [93, 106]  |
| Lipid II                          | Native phage tail-like particles | Av-CD291.2 | In vitro preclinical                                |                    | [94]       |
| Lipid II                          | Native phage tail-like particles | PL3-CD-1.74 | In vivo preclinical                                 |                    | [95]       |
| Lipid II                          | Native phage tail-like particles | MCB3681 | Phase I completed        |                    | [96]       |
| Lipid II                          | Native phage tail-like particles | MBX-500 | In vivo preclinical                                 |                    | [97]       |
| Lipid II                          | MscL              | Ramizol                              | In vivo preclinical                                 |                    | [98]       |
| Lipid II                          | Human α-defensin-5 | Virgin coconut oil                   | In vivo preclinical                                 |                    | [99]       |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [100]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | Ex vivo preclinical                                 |                    | [101]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [102]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [103]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [104]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [105]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [106]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [107]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [108]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [109]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [110]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [111]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [112]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [113]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [114]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [115]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [116]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [117]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [118]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [119]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [120]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [121]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [122]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [123]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [124]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [125]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [126]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [127]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [128]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [129]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [130]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [131]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [132]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [133]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [134]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [135]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [136]      |
| Lipid II                          | Virgin coconut oil | Combined action                      | In vivo preclinical                                 |                    | [137]      |
| Drug type                      | Mode of action       | Target            | Compound          | Investigation phase      | References |
|-------------------------------|----------------------|-------------------|-------------------|--------------------------|------------|
| Toxin-neutralizing agents     | Toxin binding        | Toxins A and B    | Tolevamer         | Phase III completed      | [153]      |
|                               |                      |                   |                   |                          |            |
|                               |                      |                   | Cholestyramine    | Phase II completed*      | [149]      |
|                               |                      |                   | Calcium aluminosilicate | Phase II terminated  | [158]      |
|                               | Premature toxin      | Toxins A and B    | IP2S4             | In vivo preclinical      | [159]      |
|                               | activation           |                   |                   |                          |            |
| Immunotherapy                 | Passive immunization | Toxins A and B    | Hyperimmune bovine colostrum | Phase II terminated | [165]      |
|                               |                      |                   | IVIG              | Phase IV terminated      | [167]      |
|                               |                      |                   | Actoxumab/bezlotoxumab | Phase III completed     | [172]      |
|                               |                      |                   | PolyCAb           | Phase I ongoing          | [175]      |
|                               |                      |                   | UCB Mab           | In vivo preclinical      | [176]      |
|                               |                      |                   | CANmAbA4/CANmAbB4 | In vivo preclinical      | [177]      |
|                               |                      |                   | Mab A2/Mab B2/Mab B1 | In vivo preclinical    | [178]      |
|                               |                      |                   | ABA               | In vivo preclinical      | [179]      |
|                               |                      |                   | VNA2-Tcd          | In vivo preclinical      | [180]      |
|                               | Active immunization  | Toxins A and B    | ACAM-CDIFF        | Phase III ongoing        | [185]      |
|                               |                      |                   | PF-06425090       | Phase II ongoing         | [187]      |
|                               |                      |                   | VLA84             | Phase II completed       | [188]      |
|                               | Toxin receptor binding sites (RBD) | Toxin A RBD | In vivo preclinical |                          | [190]      |
| Drug type | Mode of action | Target | Compound | Investigation phase | References |
|-----------|----------------|--------|----------|--------------------|------------|
| Toxin B RBD | In vivo preclinical | [191] |
| Toxin A RBD/toxin B RBD | In vivo preclinical | [192] |
| Crude SLP | In vivo preclinical | [193] |
| SlpA | In vivo preclinical | [194] |
| Cwp84 | In vivo preclinical | [195, 196] |
| FliD | In vivo preclinical | [197] |
| FliC | In vivo preclinical | [198] |
| PS-I | In vivo preclinical | [200] |
| PS-II | In vivo preclinical | [199, 201] |
| PS-III (lipotecichoic acid) | In vivo preclinical | [202] |

| Modulators of intestinal environment | Microbiota reconstitution | Intestinal microbiota | RBX2660 | Phase II completed | [218] |
|-------------------------------------|---------------------------|----------------------|---------|--------------------|------------|
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
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| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
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| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
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| SER-262 | Phase I ongoing | [223] |
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| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
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| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
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| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |
| RBX2660 | Phase II completed | [218] |
| SER-109 | Phase II ongoing | [221] |
| SER-262 | Phase I ongoing | [223] |
| MET-1 | Phase II ongoing | [225] |
| NTCD-M3 | Phase II completed | [227] |

**References**

[191] [192] [193] [194] [195, 196] [197] [198] [199, 201] [200] [202] [206–209] [218] [221] [223] [225] [227] [228] [231] [233] [234, 235, 237] [241]
in binary toxin negative strains—and the possibility of resistance emergence during CDI therapy are additional issues of concern [15–19]. Today it may only be recommended as a “chaser” therapy after vancomycin treatment to reduce CDI recurrence risk [20], based on the results of case series [19, 21] and a small RCT [22]. A larger phase IV RCT to verify these data is currently recruiting participants [23].

Rifalazil is another rifamycin derivative, which conferred significantly lower mortality—both administered prophylactically and therapeutically—in a hamster CDI model, as compared to vancomycin [24]. However, no studies on its efficacy in human CDI have been published until now.

**Other Agents**

Kibdelomycin is a novel inhibitor of type II and IV topoisomerases developed by Merck, which possesses high in vitro activity against various *C. difficile* strains [25]. Encouraging results were obtained with it in a hamster model of CDI in terms of survival (80–100%), bacterial elimination (2- to 5-log reduction of *C. difficile* counts) and a practically absent enteral absorption [25]. Upcoming phase I human trials have not yet been announced.

MGB Biopharma is developing a compound, MGB-BP-3, that interferes with the transcription of *C. difficile* DNA by binding directly to certain sequences on its minor groove [26]. It reduced both vegetative cell and spore count of intestinal *C. difficile* to a greater extent than vancomycin in an animal model [26], and it was well tolerated without raising major safety issues in a recent phase I human trial [27]. The initiation of a phase II trial with this compound is one of the major immediate objectives of the company [28].

OPS-2071 is a quinolone-based investigational compound under development by Otsuka Pharmaceutical. After two recently
completed phase I trials [29], a phase II clinical trial is currently recruiting subjects to evaluate its efficacy and safety in CDI patients [30].

Inhibitors of Protein Synthesis

**Tetracyclines**

Tigecycline is the first member of the novel glycylcycline antibiotic family with a broad antimicrobial spectrum that inhibits protein synthesis by blocking the entry of aminoacyl-tRNA into the ribosome by binding to the 30S subunit [31]. Apart from possessing activity against a wide range of gram-positive and gram-negative bacteria, including multi-resistant strains, tigecycline inhibits both toxin production and sporulation of *C. difficile* in vitro [32, 33]. Although due to its wide spectrum tigecycline may alter the intestinal microbiota significantly, thus potentially facilitating primary and recurrent CDI [34], there are various reports about its efficacy as an anti-CDI agent in both animal studies [35] and the real-life clinical setting [36–40]. Solid evidence originating from RCTs, however, is still lacking. Consequently, the place of tigecycline in the CDI treatment hierarchy is still dubious, but it may be considered as a good substitute for other broad-spectrum antibiotics in appropriate cases when withdrawal of concomitant systemic antibiotic treatment at CDI diagnosis is not possible.

Omadacycline is a new aminomethylcycline antibiotic that binds to the tetracycline binding-site of the bacterial 30S ribosome subunit, and it has considerable potency against gram-positive bacteria [41]. It also demonstrated high in vitro activity against *C. difficile* and was superior to vancomycin in terms of survival in a hamster model of CDI [42]. Its clinical evaluation in human CDI has not yet been undertaken.

**Other Agents**

Fusidic acid is an inhibitor of elongation factor G that can act in a bacteriostatic or bactericidal manner depending on its concentration and exhibits good in vitro activity against *C. difficile* [43]. It was compared with vancomycin and metronidazole in an RCT [44] and with metronidazole in another one [45], showing comparable efficacy to its comparators in both studies. In one of these trials, however, significantly higher CDI recurrence rates were observed in patients receiving fusidic acid treatment than in the vancomycin and metronidazole groups [44]. Another concern with regards to fusidic acid is the relatively frequent emergence of resistant *C. difficile* strains during treatment, which also limits its routine use [46].

A semisynthetic thiopeptide antibiotic, LFF571, interferes with bacterial protein synthesis by inhibiting the delivery of aminoacyl-tRNA to the ribosome. Its average MIC values against *C. difficile* fall between the ones observed with fidaxomicin and vancomycin [47, 48], but it does not seem to be affected by mutations conferring resistance to these antibiotics [49]. It was tested against and proved superior to vancomycin in a hamster model of CDI in terms of survival and recurrence [50], and it was non-inferior to vancomycin in a phase II human study [51]. Novartis, the company behind LFF571, has not announced forthcoming phase III trials with this compound for the time being.

CRS3123 (previously REP3123) is an experimental drug against CDI with a novel mechanism of action, namely the inhibition of *C. difficile* methionyl-tRNA synthetase, which is an essential enzyme for bacterial protein synthesis.
synthesis. It proved to be highly active against *C. difficile* without significantly affecting major representatives of the normal intestinal microbiota [52, 53]. The superiority of CRS3123 to vancomycin and metronidazole was demonstrated in terms of inhibition of toxin formation and sporulation in vitro and also in terms of survival in an in vivo model [54]. According to the developer, Crestone Pharma, phase I studies with CRS3123 are currently underway [55].

RBx 11760, a biaryl oxazolidinone, was synthesized by Ranbaxy Research Laboratories. It inhibits sporulation and also has a considerable effect on *C. difficile* toxin production [56]. In an in vivo model of CDI the hamsters treated with RBx 11760 had longer survival than the ones receiving vancomycin or metronidazole [56].

RBx 14255 is a new ketolide antibiotic developed by the same company as RBx 11760. It showed similar in vitro activity against *C. difficile* as vancomycin and metronidazole, but proved superior to both of them in terms of survival in an animal model [57].

Since Ranbaxy merged into Sun Pharmaceutical Industries in 2015, the future fate of these last two investigational compounds is unsure.

Antisense antibiotic therapy is a novel antimicrobial treatment method that aims to block the expression of key microbial genes by means of the binding of single-stranded oligomers to the corresponding complementary mRNA of the microorganism [58]. A recent study demonstrated the efficacy of this novel therapy against *C. difficile* in vitro [59]. The authors observed significant inhibition of bacterial growth with the addition of phosphorothioate gapmer antisense oligonucleotides complementary to *C. difficile* mRNAs coding essential bacterial enzymes [59]. This revolutionary treatment method has been tested in various animal models of bacterial infections, but clinical trials have only been undertaken in viral infections until today [58].

**Inhibitors of Metabolic Pathways**

Nitazoxanide is a thiazolide antibiotic by Romark Laboratories, which interferes with the metabolism of anaerobic microorganisms by inhibiting the pyruvate:ferredoxin oxidoreductase [60]. This compound possesses potent antiparasitic and antiviral properties [61] and also showed potent activity against *C. difficile* in vitro, including strains with reduced metronidazole susceptibility [62, 63]. Clinical trials comparing its efficacy with metronidazole (phase II) [64] and vancomycin (phase III) [65], however, were not conclusive, and the company presently pursues indications other than CDI for this drug [66].

Amixicile, a derivative of nitazoxanide with enhanced water solubility, synthesized at the University of Virginia, was comparable in terms of clinical cure to vancomycin and fidaxomicin in a mouse CDI model and resulted in lower recurrence rates than its comparators [67]. It does not appreciably affect intestinal microbiota, and though a considerable proportion of orally administered amixicile is intestinally absorbed, it seems to have a more favorable toxicity profile than nitazoxanide [68]. Human studies have not been undertaken with amixicile until today.

**Inhibitors of Cell-Wall Formation**

**Glycopeptides**

Glycopeptide antibiotics interfere with bacterial peptidoglycan synthesis and consequently
inhibit cell wall formation. Apart from vancomycin there are a number of newer members of this family that have been tested in the treatment of CDI with promising results. Teicoplanin may be a good alternative to vancomycin treatment, although save for a recent observational study [69] no clinical evidence has been published on its use in the last 20 years. Theoretically teicoplanin may even be superior to vancomycin in this setting since it was reported to have lower MIC values for *C. difficile* [70, 71] and patients treated with teicoplanin had notoriously lower recurrence rates than the ones receiving vancomycin in the few RCTs conducted [44, 72, 73]. This difference was not statistically significant in any of these studies, but it is to be borne in mind that all of them had a rather small number of participants. A Cochrane meta-analysis, on the other hand, showed significantly better bacteriologic cure and a close-to-significant advantage in clinical cure in patients treated with teicoplanin as compared to the ones receiving vancomycin treatment [74]. Teicoplanin has had a licensed indication for CDI since 2013, and its oral formulation is available in many countries outside the USA [1].

Ramoplanin also has lower MICs against *C. difficile* than vancomycin [75] and maintains its activity even against strains with reduced vancomycin susceptibility [76]. Its sporicidal effect is superior to vancomycin as has been demonstrated both in vitro and in vivo [77, 78]. A phase II study conducted more than 10 years ago is the only experience with ramoplanin in human subjects published [79], though according to the website of its current developer (Nanotherapeutics) a phase III RCT has already been completed and a phase IIb trial to evaluate its efficacy in CDI relapse prevention is imminent [80].

A novel lipoglycopeptide, oritavancin, showed 2–4 times lower MIC values against *C. difficile* as compared with vancomycin in an in vitro study [81]. It was tested against vancomycin in a human gut model with similar efficacy in the initial reduction of toxin levels, but oritavancin impeded recurrence after drug withdrawal to a greater extent than vancomycin [82, 83]. In a hamster model it also proved superior to vancomycin in the prevention of CDI [84]. It seems that the capacity of oritavancin to avoid CDI recurrence lays in its adhesion to dormant *C. difficile* spores, promptly eliminating cells at an early stage of germination [85]. Human studies with oritavancin in the treatment of CDI are, however, still lacking.

Demethylvancomycin is a glycopeptid antibiotic clinically used in China since the 1960s [86]. Certain N-substituted demethylvancomycin derivatives showed enhanced activity as compared to vancomycin against *C. difficile* strains in an in vitro model [87], though these findings have not been verified in in vivo models.

**Other Agents**

A number of post-translationally modified bacteriocins (lantibiotics) have been subjects of research in the laboratories of Novacta Biosystems in recent years, which showed fair capacity to inhibit cell-wall synthesis. Actagardine, though it is not very active against *C. difficile* in itself, in combination with ramoplanin showed a potent synergistic effect against the majority of *C. difficile* strains tested in vitro [88], and a mutant variant of it, V15F, seems to be twice as active as actagardine [89]. NVB302 proved to be non-inferior to vancomycin in a human gut model of CDI [90], and a successful phase I trial has also
already been completed with this compound [91].

A synthetic analog of the naturally occurring nucleoside-based capuramycin, SQ641, targets translocase-1, an essential enzyme in bacterial cell-wall synthesis. It was originally intended to be an anti-tuberculosis agent, but since it had good in vitro activity against *C. difficile* it was tested in a mouse model of CDI and was superior to vancomycin in terms of 14-day survival [92]. The company behind this compound, Sequella, has not announced forthcoming human studies for the time being.

**Agents with Direct Effect on Cell Wall/Cell Membrane**

Surotomycin (MK-4261, previously CB-183,315) is a membrane-active cyclic lipopeptide, whose rights are owned by Merck since its acquisition of the original developing company, Cubist Pharmaceuticals, in 2015. It has potent bactericidal activity against both growing and stationary phase *C. difficile* [93]. It is also active against *Enterococci* [94] and disrupts intestinal microbiota only moderately [95, 96], although this may be sufficient to bring forward the overgrowth of extended-spectrum beta-lactamase-producing *Klebsiella* in surotomycin-treated patients [97]. On the other hand, the emergence of resistant *C. difficile* or *Enterococcus* strains during treatment is possible but fairly unlikely [98, 99]. In a phase II clinical trial CDI recurrence rates were significantly lower and sustained clinical response rates were significantly higher in patients treated with surotomycin as compared to the vancomycin-treated control group [100]. However, the results of one of the two recently completed phase III trials [101] were recently made public, according to which the non-inferiority of surotomycin to vancomycin could not be demonstrated [102]. These disappointing results may be the explanation for the absence of surotomycin in Merck’s latest research pipeline chart [103].

Thuricin CD, a sactibiotic produced by *Bacillus thuringiensis*, was proved to possess narrow-spectrum activity against *C. difficile* [104], having very little effect on the rest of the normal intestinal microbiota [105]. According to a recent study, the combination of Thuricin CD with other antiClostridial antibiotics may have a synergistic effect on biofilms formed by certain *C. difficile* strains [106].

*Clostridium difficile* produces so-called phage tail-like particles (PTLPs) that successfully eradicated *C. difficile* in an in vitro model [107]. In another study, administration of a genetically modified PTLP (Avidocin CD), called Av-CD291.2, successfully prevented *C. difficile* colonization in mice without interfering with the normal colonic flora [108]. The company behind Av-CD291.2, AvidBiotics, is planning to start human studies with this compound in the near future [109].

PLYCD1-174 is the recombinantly expressed catalytic domain of a prophage endolysin identified in a *C. difficile* strain (CD630). It exerted potent lytic activity against *C. difficile* without significantly affecting other components of the intestinal microbiota in an ex vivo mouse model [110].

**Hybrid Antibiotics and Other Agents with Multiple Mechanisms of Action**

Cadazolid is a new fluoroquinolone-oxazolidinone antibiotic by Actelion, which exerts its antibacterial effect principally by the inhibition of protein synthesis and to a lesser degree also interferes with bacterial DNA synthesis [111]. Cadazolid not only has lower MICs against *C. difficile* than vancomycin and
even fidaxomicin [112, 113], but it is also highly active against vancomycin resistant Enterococci, a major threat in vancomycin-treated patients, minimizing the risk of patient colonization by this much-dreaded bacteria [114]. This, combined with its potent activity against toxin formation and sporulation, makes it a promising new recruit among anticostridial drugs. It performed well in a phase II trial as compared to vancomycin in first episodes or first recurrences of CDI [115], and it is currently being tested in two phase III clinical trials [116].

MCB3681, a novel fluoroquinolone-oxazolidinone antibiotic by Morphochem, also proved to possess excellent in vitro efficacy against C. difficile [117]. In a phase I study its water-soluble prodrug, MCB3837, was administered intravenously to healthy individuals and showed a marked reduction of gram-positive organisms without considerably affecting the gram-negative portion of intestinal microbiota [118]. Based on these data the Food and Drug Administration recently granted Fast Track designation to the compound, and the start of a phase II clinical trial with MCB3837 seems imminent [119].

MBX-500 (a.k.a compound 251D) is a novel hybrid antibiotic that binds to three different bacterial targets: its anilinuracil component acts as a DNA polymerase inhibitor, and its fluoroquinolone component blocks DNA topoisomerase and gyrase enzymes [120]. Its multiple-site action seems to make the rise of resistant strains during treatment less probable [120]. The in vivo efficacy of MBX-500 against C. difficile was demonstrated in at least three animal CDI models (hamsters [121], mice [121] and piglets [122]), but human studies have not yet been announced by its developer, Microbiotix.

Ramizol is the first member of a new antibiotic family that selectively targets the mechanosensitive ion channel of large conductance (MscL) in bacteria [123] and possesses potent antioxidant properties as well [124]. In a hamster model of CDI orally administered Ramizol conferred lower 28-day survival than vancomycin (57% vs. 86%), and its spore-clearing capacity was also inferior to that of vancomycin (29–57% vs. 100%) [125]. The company behind it (Boulos and Cooper Pharmaceuticals), however, is currently working on a formulation of enhanced efficiency of this drug [125] and expects to initiate phase I clinical trials in 2017 [126].

Human α-defensin 5 (HD5) is an enteric representative of membrane-active antimicrobial proteins and peptides (AMPs) produced in the small intestine by Paneth cells, forming an important, though not yet truly understood role in host-microbiota interactions [127]. Its role in C. difficile toxin-neutralization was suggested by one study [128], and direct C. difficile cell killing was observed in another one with physiological concentrations of HD5 [129]. Whether these characteristics of HD5 may be exploited in the fight against C. difficile is unclear.

Certain medium-chain fatty acids, such as lauric acid derived from virgin coconut oil, caused a significant inhibition of C. difficile growth in an in vitro study [130]. It is postulated that bacterial cell death is caused by the incorporation of these fatty acids in the bacterial cell membrane that brings forth a substantial change in its permeability as well as by a probable interference with cellular metabolism [131]. In vivo studies on the efficacy of virgin coconut oil or its derivatives in the prevention or treatment of CDI have not yet been undertaken.
Agents of Unknown/Unclear Mechanism of Action

Ridinilazole (previously SMT19969) is a narrow-spectrum antibiotic with poor oral bioavailability that exhibits 2–17 times lower MIC values against *C. difficile* than vancomycin [132–134]. Its precise mechanism of action is not yet fully known, but it had a potent bactericidal effect on *C. difficile*, caused significant reduction of toxin levels and also demonstrated anti-inflammatory activity in vitro on human intestinal cells [135]. In a hamster model, better 28-day survival rates were observed in animals treated with ridinilazole than in the ones given vancomycin or fidaxomicin [136]. A phase II RCT was completed recently, where ridinilazole achieved the non-inferiority goal set in terms of clinical cure and was superior to vancomycin in terms of sustained clinical response after 30 days of follow-up [137]. Summit Therapeutics is currently preparing phase III RCTs with this compound [138].

Berberine is a natural substance already utilized in ancient Chinese folk medicine. The addition of berberine to vancomycin significantly improved mortality, prevented weight loss and decreased recurrence rates in a mouse model of CDI [139]. The authors speculated that berberine may counteract the deleterious effect of vancomycin on intestinal microbiota by inhibiting the expansion of *Enterobacteriaceae*. A recent in vitro study, however, showed a high berberine uptake of germinating *C. difficile* spores where it may reach elevated concentrations, inhibiting spore outgrowth on its own [140].

The bacteriostatic effect of bovine lactoferrin on *Clostridium* species has been known for more than 20 years [141]. As a biomarker for intestinal inflammation, its fecal concentrations seem to correlate with CDI severity [142]. Although it is postulated that lactoferrin exerts its bacteriostatic activity by acting as an iron-sequestering agent, in a recent mouse model of CDI the administration of iron-saturated—but not iron-depleted—bovine lactoferrin successfully inhibited *C. difficile* toxin production and delayed *C. difficile* outgrowth, without significantly affecting the rest of the intestinal microbiota [143]. A phase II RCT on its efficacy in the prevention of antibiotic-associated diarrhea in children is currently underway [144].

A completely new approach is being investigated under the aegis of Photobiotics Ltd., which aims to eradicate *C. difficile* by means of radical oxygen species produced by light-activated photosensitizers. The method, called Photodynamic Antimicrobial Chemotherapy (PACT), involves the activation of a light-sensitive dye (photosensitiser) by visible light that utilizes the received energy to generate radical species or singlet oxygen that, in turn, leads to cell death [145]. In an in vitro model, 99.9% of *C. difficile* colonies was eliminated by this method without causing appreciable harm to surrounding colon cells [145], but in vivo studies have not been conducted so far.

TOXIN-NEUTRALIZING AGENTS

Unlike the agents discussed above, toxin-neutralizing drugs aim not to eliminate *C. difficile*, but to prevent its cytotoxic effect on colonocytes. These agents either sequester or inactivate *C. difficile* toxins impeding them from reaching their target cells, hence preventing or alleviating the clinical manifestations of CDI. Their use as prophylactic or adjuvant therapy has a solid scientific basis, but since they have no effect on
either vegetative *C. difficile* or its spores they do not prevent asymptomatic carriage and transmission.

**Toxin Binding**

Cholestyramine, an ion exchange resin usually used as a bile acid sequestrant, was the first one of the agents in this category to be tested more than 35 years ago [146]. There were various cases of recurrent CDI reported to respond to cholestyramine treatment by that time [147, 148], but the compound was never tested in any formal clinical trial. It does not seem to be suitable as an adjunct therapy to vancomycin, since it not only binds *C. difficile* toxins but the antibiotic as well [70]. Based on the encouraging results of a recent pilot study it was proposed to be used as primary CDI prophylaxis in patients receiving long-term systemic antibiotic treatment [149], but these findings will have to be confirmed in larger RCTs.

Tlevamer, a toxin-binding polymer, was a compound of great expectations during the first decade of this century [150, 151]. The initial enthusiasm, however, flagged considerably in light of more recent studies that proved its toxin neutralization capacity to be much lower than previously thought [152], and its clinical efficacy failed to match that of vancomycin or metronidazole in two clinical trials [153]. In these trials, however, CDI recurrence rates were significantly lower in the subset of patients that responded to tolevamer than in the subsets of responders in the vancomycin and the metronidazole group [154]. These data may adumbrate a potential use of tolevamer in CDI prophylaxis in the future, although Genzyme (now Sanofi Genzyme) indefinitely halted its development after the unsuccessful trials.

Calcium aluminosilicate anti-diarrheal (CASAD) is a naturally occurring clay known to possess considerable cation exchange absorbent properties. Its potential as a prophylactic agent in cancer-related diarrhea was recently investigated by Salient Pharmaceuticals, with inconclusive results [155, 156]. However, because of its in vitro capacity to sequester *C. difficile* toxins [157], a phase II trial was initiated to investigate its efficacy as an adjuvant therapy of CDI, which was prematurely terminated because of slow enrollment [158].

**Premature Toxin-Activation**

*Clostridium difficile* takes advantage of a host cytosolic enzyme, inositol hexakisphosphate, that triggers an autocleavage process of the toxins once these become endocytosed into colonocytes, which results in their activation and the subsequent cytotoxic effect. A novel approach endeavors to combat CDI by the premature luminal activation of *C. difficile* toxins using an inositol hexakisphosphate analog (INS-5010) where some of the phosphate groups were substituted by sulfate groups for stability purposes. *C. difficile*-infected mice treated with this compound showed a significant reduction of histological signs of colitis as compared to control subjects [159]. The Swiss company developing INS-5010, Inositec AG, has not yet announced the initiation of human studies [160].

**Passive Immunization**

The potential of colostrum of pregnant cows immunized with *C. difficile* toxoids (hyperimmune bovine colostrum, HBC) to inhibit the cytotoxic and enterotoxic effect of *C. difficile* toxins has been known for more than 2 decades [161, 162]. Recently, it was proved to alleviate symptoms of CDI in gnotobiotic
piglets without detectably affecting normal intestinal microbiota [163]. Whey protein concentrate derived from immunized cow milk was proved to be safe and well tolerated in human subjects with CDI as well [164]. A randomized phase II study comparing the efficacy of immune whey with metronidazole in the treatment of recurrent CDI showed similar clinical results to the two treatment modalities, but was terminated prematurely because of the bankruptcy of the sponsor (Novatreat Ltd.) [165].

The use of intravenous immunoglobulin (IVIG) in CDI has been subject to discussion for decades, but in the absence of evidence from RCTs its potential benefits remain controversial [166, 167]. There are only two relatively small retrospective matched cohort studies published that compare the clinical efficacy of the addition of IVIG to conventional anti-clostridial treatment [168, 169]. Neither of them found significant differences between the compared cohorts in the main clinical outcomes, though the authors of one of these studies point out that in their IVIG cohort there were significantly older patients with more severe CDI than in the control group [169].

The results of the first study on the efficacy of monoclonal antibodies against *C. difficile* toxin A (actozumab, previously MK3415) and toxin B (bezlotoxumab, previously MK6072 and MDX1388) in a hamster model of CDI were published exactly 10 years ago [170]. In that study enhanced efficacy was observed with the combination of the two antibodies in terms of both recurrences and mortality. In a phase II trial with this combination (a.k.a. MK-3415A), the addition of the neutralizing antibodies to standard CDI treatment also significantly lowered recurrence rates [171]. However, although the efficacy of bezlotoxumab in preventing CDI recurrence was confirmed in two recently conducted phase III trials, its combination with actozumab, surprisingly, did not show any additional benefit in these studies [172]. Based on these results the US Food and Drug Administration (FDA) has very recently approved bezlotoxumab, and it will be available in the first quarter of 2017 [173].

There are several other companies and research entities as well focusing on monoclonal antibodies against *C. difficile* toxins. A mixture of anti-A and anti-B neutralizing antibodies of ovine origin (PolyCAB) is under research by MicroPharm, which showed a significant reduction of CDI recurrence in a hamster model [174]. Phase I trials with this product are currently ongoing [175]. UCB Pharma financed an investigation with UCB MAb, a mixture of three humanized antibodies (two against toxin A and one against toxin B) that proved to be superior in terms of survival after 28 days as compared to bezlotoxumab in hamsters [176]. Another mixture of humanized anti-A and anti-B antibodies (CANmAbA4 and CANmAbB4) by Emergent BioSolutions recently demonstrated excellent in vitro toxin neutralizing capacity and also conferred protection against clinical CDI in a hamster model [177]. BliNK Biomedical SAS, in turn, is developing a cocktail consisting of an anti-A and two anti-B monoclonal antibodies of entirely human origin (MAb A2 + MAb B1 + MAb B2) with very promising results in an animal study published last year [178]. A novel bispecific single-domain antibody, referred to as ABA, with binding domains for both toxin A and B demonstrated excellent toxin neutralization activity in vivo and achieved a 100% survival in mice with fulminant CDI caused by the hypervirulent strain BI/NAP1/027 [179]. A modified version of ABA, VNA2-Tcd, has recently shown an outstanding protective...
capacity against CDI in another animal model [180]. Apart from these, various other monoclonal or single-domain antibodies against the toxins or other structural components (surface-layer proteins, flagella, etc.) of *C. difficile* are subjects of investigation currently, but none of these are in the clinical (and most of them not even in in vivo preclinical) phase of development yet [181].

**ACTIVE IMMUNOTHERAPY**

The preparation of the host immune system for a potential encounter with *C. difficile* and its toxins is the objective of this therapy. The most advanced vaccines elicit a potent immune response against toxins A and B, but—similarly to toxin-neutralization—they do not lower colonization rates, and their preventive value manifests itself only on an individual level as the transmission risk remains unaffected. Other interesting vaccine candidates—which are in much earlier stages of development—targeting different surface components of *C. difficile* may offer certain advantages in this sense in the future.

**Toxoid and Recombinant Toxin-Based Vaccines**

ACAM-CDIFF is a toxoid vaccine against both toxin A and toxin B of *C. difficile* developed by Sanofi Pasteur. After positive immunological and clinical results obtained in a hamster model [182] and in two phase I studies [183], ACAM-CDIFF recently passed a phase II trial without any safety issues [184]. A phase III RCT with this vaccine is currently ongoing [185].

Pfizer is developing another toxoid vaccine (PF-06425090) with good results obtained in a phase I clinical trial [186], and two phase II trials with this product are currently under way in healthy adults [187].

VLA84 (a.k.a. IC84), a recombinant protein vaccine containing epitopes of toxin A and toxin B developed by Valneva, showed positive results in a recent phase II trial in terms of safety, tolerability and immunogenicity in both younger adults and elderly volunteers [188]. The vaccine is soon to be tested in a phase III RCT, according to the company [189].

**DNA Vaccines**

DNA vaccines have the theoretical advantage over conventional vaccination of inducing not only humoral but also cellular immune response. The first DNA vaccine candidate for *C. difficile* encoded the receptor-binding site (RBD) of *C. difficile* toxin A [190]. Inoculated mice produced a potent antibody response to toxin A, and up to 100% survival was observed (depending on vaccine formulation and mouse strain) after challenge with a lethal dose of toxin A [190].

In another mouse model, apart from the RBD of toxin A, the N-terminal enzymatic domain of toxin B also showed a positive antibody response [191]. The combination of the antibodies elicited by these two DNA vaccines conferred 100% protection against *C. difficile* in the experimental animals [191].

More recently, promising results were published with a novel DNA vaccine that contains plasmids encoding optimized RBDs of both toxin A and B of *C. difficile* [192]. The sera of immunized animals (mice and primates) showed potent toxin neutralization ability in vitro, and the combined vaccine provided a 50–90% protection against a lethal dose of *C. difficile* spores in mice [192].

△ Adis
Vaccines Against *C. difficile* Surface Antigens

Although toxin-based vaccine candidates may successfully prevent toxin-mediated tissue damage and lessen the clinical manifestations of CDI, they do not prevent carrier status because they have no effect on *C. difficile* colonization. Vaccines targeting *C. difficile* surface antigens (proteins or carbohydrates) aim to offer a solution to this problem.

The surface layer protein (SLP) is the major surface antigen of *C. difficile*. It is made up of an outer layer formed by low-molecular-weight SLP (LMW-SLP) and an inner layer formed by high-molecular-weight SLP (HMW-SLP). These two proteins are created from a common precursor molecule (SlpA) that undergoes enzymatic cleavage by a protease (Cwp84) once SlpA reaches the cell surface. Intranasal or intraperitoneal administration of crude SLP (containing both HMW-SLP and LMW-SLP) conferred only moderate protection against a lethal *C. difficile* challenge in hamsters and mice [193]. Intra-rectal vaccination with the precursor protein SlpA in another animal study resulted in slightly more favorable results, as it significantly decreased the colonization level in the vaccinated mouse group, though it also failed to provide significant benefit in terms of survival [194]. The immunogenicity and protective ability of the protease Cwp84 have also been investigated in hamsters: rectally administered Cwp84 partially inhibited intestinal colonization and significantly increased the survival of hamsters challenged with *C. difficile* in one study [195], and similar survival rates (40%) were observed in another one with the intragastric administration of the same vaccine encapsulated in pectin beads [196].

Elements of *C. difficile* flagellum can also be found among potential future vaccine candidates. Recombinant flagellar cap protein (FlID) in combination with either Cwp84 or a mix of other flagellar proteins administered rectally to mice resulted in significantly lower colonization rates as compared to control animals [197]. Intraperitoneal vaccination with recombinant flagellin (FlIC), on the other hand, significantly improved survival in mice and hamsters infected with *C. difficile* in a recent study, while leaving the rest of the intestinal microbiota apparently intact [198].

All three known surface polysaccharides of *C. difficile* have been proved to be capable of eliciting a certain level of immune response. PS-I was first detected on the hypervirulent *C. difficile* strain 027 but it is not specific to this ribotype [199]. Conjugates of either synthetic PS-I or its disaccharide minimal immunogenic epitope with the diphtheria toxin variant CRM197 proved immunogenic in mice [200]. Another murine model demonstrated the immunogenicity of a conjugate vaccine of a synthetic hexasaccharide epitope of PS-II with CRM197 [201], and PS-II-specific immune response was observed in swine as well after being vaccinated with a non-adjuvanted PSI/PSII preparation [199]. In a recent study a conjugate vaccine of lipoteichoic acid (LTA, a.k.a. PS-III) and CRM197 not only evoked antigen-specific immune response in a mouse model, but also significantly inhibited intestinal *C. difficile* colonization [202].

There are a number of other potential *C. difficile* vaccine candidates in the preclinical phase of investigation that may be tested in the clinical setting as well in the forthcoming years [203, 204].
RESTORATION AND MODULATION OF THE INTESTINAL MICROBIOTA

Preservation and/or restoration of colonization resistance of the intestinal microbiota is the primary objective of the following therapies with subsequent protection against toxigenic *C. difficile* colonization or its elimination from the colon. Among these methods there are some that endeavor to offer the most complete solution for the problem of CDI, aiming to achieve clinical cure and the prevention of carriage, transmission and recurrences all at once.

Microbiota Restoration

The microbiota-restoring therapy par excellence is intestinal microbiota transplantation, which has been living a true renaissance in the last years. Its excellent clinical efficacy is further upheld by the observation that host intestinal microbiota composition and diversity are intimately linked to CDI severity and recurrence risk [205].

That the restoration of an impaired intestinal microbiota by transplanting feces of a healthy individual may effectively cure CDI and prevent recurrences is not questioned any more [1, 20]. Its efficacy was proven in two already published phase II RCTs [206, 207], and another phase II RCT performed on pediatric patients has also recently been completed [208], whose results are not yet available. Beside these completed studies there are various ongoing phase II and III RCTs with this treatment method [209].

With the demonstration that the efficacy of previously recollected frozen and freshly harvested microbiota have the same efficacy, intestinal microbiota transplantation has become a lot less cumbersome than before [210, 211]. However, the manipulation of feces and classical enteral administration methods (i.e., by nasogastric tube, rectal enemas or via colonoscopy) are not only laborious, but make this procedure rather unattractive for physicians and patients alike [212–214]. For this reason there are a number of efforts being made to enhance feasibility and social acceptance of this highly efficient therapy. Oral administration of capsulized intestinal microbiota is one of the pioneer solutions for the above problem [215]. The elevated number of microbiota-containing capsules to be administered, though, is a major drawback of this method, which may be improved by submitting microbiota to lyophilization instead of simple freezing [216, 217].

RBX2660 is a commercially available, standardized microbiota suspension prepared from human stool (Rebiotix Inc.) that demonstrated its efficacy in a multicenter open-label phase II study [218]. A phase IIb RCT with this product has recently completed enrollment, and if its results are favorable RBX2660 is intended to enter phase III [219].

Seres Therapeutics has recently developed SER-109, which contains around 50 species of feces-derived *Firmicutes* spores after the elimination of the rest of the microbiota with ethanol. In an uncontrolled study, it was administered orally in an encapsulated form to 30 patients with multiple CDI recurrences after an appropriate response of standard of care antibiotic treatment, and no further recurrences were observed during the 8-week-long follow-up in 96.7% of the subjects [220]. A phase II trial to compare its efficacy with placebo is currently recruiting participants [221]. The same laboratory is investigating another microbial preparation comprised of spores of 12 different bacterial species (SER-262) meant to prevent recurrence after first CDI episodes [222]. An ongoing phase Ib
RCT is currently evaluating the efficacy of this product as compared to placebo [223].

MET-1 (Microbial Ecosystem Therapeutic-1) is a defined intestinal bacterial culture originating from a single donor. Two patients with recurrent CDI who were treated with this product experienced clinical cure in 2–3 days and remained recurrence free for the 6 following months [224]. An open-label pilot study is about to be initiated to demonstrate its efficacy in recurrent CDI in comparison with vancomycin [225].

The administration of nontoxigenic C. difficile strains is another original approach to prevent colonization by toxigenic C. difficile. The most advanced research is being done with the nontoxigenic C. difficile strain M3 (NTCD-M3, a.k.a. VP20621), with encouraging results in animals [226] and in a recent phase II placebo-controlled human trial as well [227]. Another nontoxigenic strain, CD37, also provided significant protection against CDI in a mouse model as observed by less weight loss and less mortality in mice treated as compared to untreated animals [228].

### Bile Acid Therapy

The bile acid composition of the gut has an important role in the upholding of colonization resistance, as certain secondary bile acids formed by members of a healthy microbiota have an important role in the inhibition of C. difficile spore germination [229]. The loss of this effect due to the disruption of intestinal microbiota by broad-spectrum antibiotic treatment is a key factor in C. difficile outgrowth, potentially leading to clinical CDI [230]. A secondary bile acid, ursodeoxycholic acid, was recently proved beneficial in a case of refractory C. difficile-associated pouchitis [231], but further evaluation of this compound in this setting has not yet been undertaken. A taurocholate analog, CamSA, is a potent in vitro inhibitor of C. difficile germination [232] that successfully prevented CDI in mice in a dose-dependent manner [233]. Whether secondary bile acids or their analogs will play a role in CDI prevention or if they can also be beneficial as adjunct in CDI treatment is still unknown.

### Intestinal Antibiotic Inactivators

CDI risk is intimately linked to the damage systemic antibiotic therapy exerts on the intestinal microbiota. Methods to avoid this deleterious side effect by the inactivation of antibiotics that reach the colon lumen promise not less than the elimination of this single major risk factor in cases when antibiotic treatment is inevitable.

DAV132 is an activated-charcoal-based product with enteric coating that successfully adsorbs antibiotics such as amoxicillin or moxifloxacin that reach the proximal colon [234, 235]. This compound, developed by DaVolterra, proved to prevent moxifloxacin-induced CDI in an animal model [236] and was demonstrated to be safe and efficient in humans as well, in two phase I RCTs [234, 235]. A dose-finding phase I RCT is currently recruiting participants [237], and a phase II trial in patients at risk of CDI is already being prepared by the company [238].

The capacity of orally administered beta-lactamase enzymes to inactivate parenterally administered beta lactam antibiotics secreted into the gut is the basis of another approach to prevent antibiotic-induced microbiota damage [239]. SYN-004 is a recombinant beta-lactamase developed by Synthetic Biologics that efficiently inactivated intravenous ceftriaxone in the gut of dogs.
without interfering with serum antibiotic levels [240]. Its efficacy and good tolerability were recently proved by two phase I RCTs [241], and three phase II trials are currently underway with this product [241].

The same effect is sought by the research group that colonized the gut of mice with cephalosporinase-producing Bacteroides thetaiotaomicron to evaluate its efficacy in inactivating the intestinally excreted portion of subcutaneously administered ceftriaxone [242]. The investigators observed that the cephalosporinase produced by B. thetaiotaomicron successfully preserved normal intestinal microbiota and prevented colonization by both C. difficile and vancomycin-resistant Enterococcus in treated mice.

ENTEROPROTECTIVE AGENTS

These compounds are not explicitly anti-clostridial agents, but seek to alleviate the inflammation of the colon in CDI patients, hence decreasing morbidity and improving survival of severely ill patients.

Glutamine supplementation is traditionally used to preserve and/or restore intestinal mucosal integrity in patients with prolonged parenteral nutrition, gastric ulcer, chemotherapy, radiotherapy or intestinal surgery, among other conditions. Alanyl-glutamine is a bipeptide derived from glutamine with enhanced stability and water solubility. Its capacity both in vitro and in vivo to counteract cytotoxic activity of C. difficile toxins A and B was demonstrated in different studies [243, 244]. In a mouse CDI model the addition of alanyl-glutamine to vancomycin treatment resulted in improved survival as compared to vancomycin treatment alone [244]. There is currently a phase II human trial underway at the University of Virginia to test the efficacy of alanyl-glutamine supplementation in reducing mortality and recurrences in CDI patients receiving standard antclostridial treatment [245].

The activation of adenosine A2A receptors in macrophages and neutrophils has anti-inflammatory and tissue-protective effects by regulating the secretion of pro- and anti-inflammatory cytokins. The combination treatment with vancomycin and an adenosine A2A receptor agonist (ATL370 or ATL1222) resulted in less weight loss and better survival in a mouse CDI model as compared with vancomycin monotherapy [246]. Another adenosine A2A receptor agonist, ATL313, successfully prevented major damage of murine ileal mucosa induced by C. difficile toxin A [247]. The combination of alanyl-glutamine and an ATL370 also efficiently reversed histopathologic damage caused by toxin A in another animal study [248]. No human studies have been conducted with these products for the time being.

CONCLUDING REMARKS

The number of potential therapies for CDI has been growing steadily recently, and this trend may continue for some years, as both the incidence and severity of C. difficile keep increasing, and current therapeutics cannot offer a definite solution for this situation. Antibiotic surveillance programs and more directed anti-clostridial antibiotics have been demonstrated to be useful, but the steadily increasing number of susceptible hosts (immunocompromised patients, elderly, etc.) may prove these efforts insufficient to halt the epidemic.
It seems clear that none of the described novel treatment methods will be able to deliver the ultimate solution for CDI single-handedly, since none of them can address all the components of the compound objective of CDI management, that is, primary prophylaxis, cure and prevention of recurrences. However, the more prophylactic and therapeutic tools are available the more efficient anti-CDI strategies can be tailored for different patient populations in different geographical areas with varying C. difficile prevalence.

Certainly, the greatest impact on CDI prevalence is to be expected from measures that aim to prevent the infection by means of active immunization or by protecting the healthy intestinal microbiota. On the other hand, the prompt restoration of a damaged microbiota and potent and narrow spectrum anti-clostridial antibiotics may guarantee a quicker recovery in established CDI, hence diminishing transmission and recurrence risk alike. At the same time, anti-toxin and anti-inflammatory treatment may improve the clinical outcome in severe and/or complicated CDI. As new therapeutic agents appear on the market, efforts shall be made to find the optimal way to combine them in a way that minimizes incidence and horizontal transmission, maximizes cure rates and keeps recurrences at a minimum.

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