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Review

Clostridioides difficile infection: are the three currently used antibiotic treatment options equal from pharmacological and microbiological points of view?

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ABSTRACT

Recently, the recommendations for the treatment of *Clostridioides difficile* infection (CDI) have been updated. However, in addition to the clinical efficacy data, the drug of choice should ideally represent optimal antimicrobial stewardship, with an emphasis on rapid restoration of the gut microbiota to minimize the risk of infection relapses. Oral administration of metronidazole results in low concentration in stool, and interaction with fecal microbiota reduces its antimicrobial bioactivity. Reported elevated minimum inhibitory concentrations of metronidazole in epidemic *C. difficile* ribotypes and the emergence of plasmid-mediated resistance to metronidazole represent additional potential risks for clinical failure. If metronidazole is the only CDI treatment option, antimicrobial susceptibility testing on agar containing heme should be performed in *C. difficile* isolate. Compared with metronidazole, oral vancomycin and fidaxomicin reach very high concentrations in the stool, and therefore can quickly reduce *C. difficile* shedding. Health care facilities with higher CDI incidence and/or occurrence of epidemic ribotypes should not use metronidazole because prolonged *C. difficile* shedding can increase the risk for further *C. difficile* transmission. Only fidaxomicin has a narrow spectrum of antimicrobial activity, which might be, together with persistence on spores, the main contributing factor to reduce the recurrent CDI rates.

1. Introduction

Clostridioides difficile is the leading cause of health care–associated diarrhea and a frequent cause of infective diarrhea in the community. *C. difficile* infection (CDI) increases in-hospital mortality and excess health care costs and has a long-lasting effect on the quality of life of the patients (Barbut *et al.*, 2019; Hensgens *et al.*, 2014; Marra *et al.*, 2020; Vent-Schmidt *et al.*, 2020).

Since 2017, various international organizations have updated their guidance documents and recommendations for CDI treatment (Johnson *et al.*, 2021; Kelly *et al.*, 2021; Krutova *et al.*, 2022; McDonald *et al.*, 2018; van Prehn *et al.*, 2021). Due to a significant reduction in recurrent rates, fidaxomicin is the preferred option in initial nonsevere CDI and the first CDI recurrence (Johnson *et al.*, 2021; Krutova *et al.*, 2022; van Prehn *et al.*, 2021). Fidaxomicin is an equal option in severe CDI compared with vancomycin (Johnson *et al.*, 2021; Kelly *et al.*, 2021; Krutova *et al.*, 2022; McDonald *et al.*, 2018; van Prehn *et al.*, 2021). All guidance documents except for one do not recommend oral metronidazole as the first-line drug for the treatment of initial nonsevere CDI and consider metronidazole use only when fidaxomicin or vancomycin is not available (Johnson *et al.*, 2021; Kelly *et al.*, 2021; Krutova *et al.*, 2022; McDonald *et al.*, 2018; van Prehn *et al.*, 2021). The American College of Gastroenterology still supports the use of metronidazole for initial nonsevere CDI in low-risk patients, such as younger

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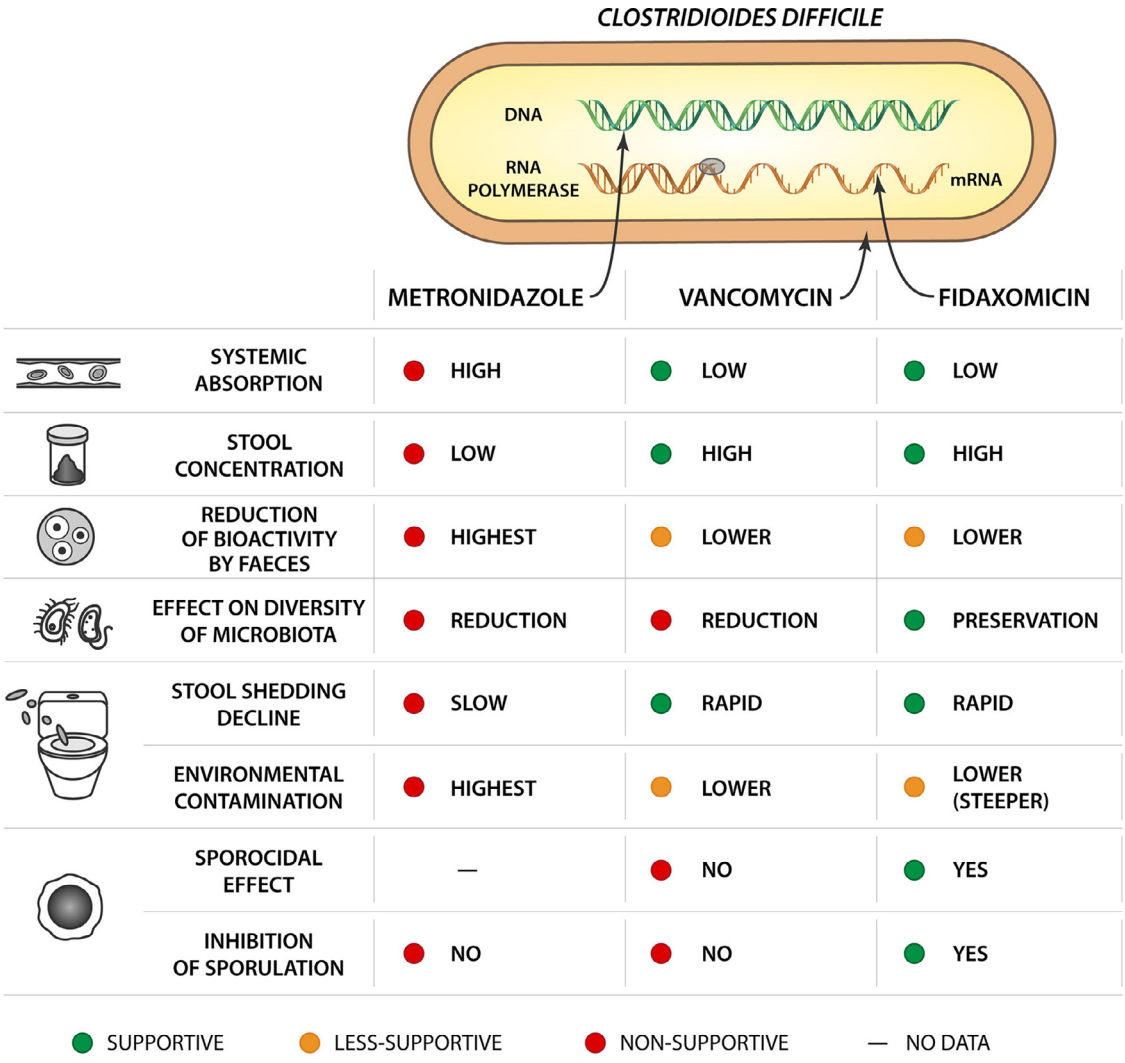

Overview of pharmacodynamic, pharmacokinetic and microbiological properties for oral administration of metronidazole, vancomycin and fidaxomicin.

Figure.

outpatients with minimal comorbidities (Kelly *et al.*, 2021). Revised practice guidelines have had a significant impact on CDI treatment with an increase in vancomycin and fidaxomicin prescription. Although the prescription of metronidazole decreased after the publishing of recommendations, it is still one of the most frequently used antimicrobials in patients with CDI (Clancy *et al.*, 2021).

Undoubtedly, antibiotic treatment of CDI already has very limited options, and these have been further reduced. In addition to clinical efficacy data, the drug of choice should be in line with good antimicrobial stewardship practice, with an emphasis on rapid restoration of the depleted gut microbiota to reduce the risk of infection relapses. The aim of this narrative review was to augment the CDI treatment recommendations by summarizing the pharmacological and microbiological properties of fidaxomicin, vancomycin, and metronidazole.

2. Literature search

The literature for this narrative review was drawn from a search of PubMed until March 2022. Index search terms were *Clostridium difficile, Clostridiodes difficile*, metronidazole, vancomycin, fidaxomicin, gut microbiota, resistance, shedding, and stool concentration. Only original studies written in English were included. The references of articles were also screened and added, if appropriate.

An overview of pharmacodynamic, pharmacokinetic, and microbiological properties for oral administration of metronidazole, vancomycin, and fidaxomicin is shown in Figure.

3. Pharmacological properties

Oral metronidazole is absorbed almost completely (90%) in the upper gastrointestinal tract and enters the large intestine primarily through secretion across the gut mucosa; the intraluminal concentrations of metronidazole are proportional to the extent of inflammation (Bolton and Culshaw, 1986; Lamp *et al.*, 1999). Metronidazole therapy (six CDI episodes with 400 mg every 8 hours orally, three CDI episodes with 500 mg every 8 hours intravenously, and one CDI episode with only 200 mg every 8 hours orally) resulted in mean levels (± SD) in watery feces of 9.3 ± 7.5 μg/g wet weight (range 0.8-24.2) decreasing to 3.3 ± 3.6 μg/g wet weight (range 0.5-10.4) in semiformed stool samples and have been found to be very low or zero (1.23 ± 2.8 μg/g; range 0-10.2) in formed stool samples (Bolton and Culshaw, 1986). Notably, the dosage in the majority of patients in this study was about 20% lower than that in

CDI treatment recommendations, and it is unknown whether stool concentration would increase noticeably when the dosage of 500 mg every 8 hours is administered.

In contrast to metronidazole, fecal concentrations of vancomycin with a dosage of 125 mg every 6 hours ranged from 175-6299 μg/g; for fidaxomicin, for a dosage of 200 mg every 12 hours, concentrations were 1396 ± 1019 µg/g, with 834 ± 617 µg/g for OP-1118, which is an active metabolite of fidaxomicin (Sears *et al.*, 2012; Thabit and Nicolau, 2015).

Unlike metronidazole, systemic absorption is minimal after oral administration of either vancomycin or fidaxomicin (Sears *et al.*, 2012; Thabit and Nicolau, 2015). However, in severe inflammation of the intestinal mucosa with concomitant renal impairment, systemic absorption of orally administered vancomycin can be enhanced (European Medicines Agency, 2017), especially with higher dosages (250-500 mg per 6 hours) (Pogue *et al.*, 2009; Yamazaki *et al.*, 2017). The increased systemic absorption due to severe inflammation can also hypothetically be expected with fidaxomicin; however, the nonclinical pharmacology and safety pharmacology of fidaxomicin has not revealed any unexpected effects overall (European Medicines Agency, 2022).

4. *In vitro* activity

4.1. *Vegetative* Clostridium difficile *cells*

Metronidazole is a nitroimidazole that inhibits DNA synthesis, with bactericidal activity against anaerobic bacteria and protozoa. Metronidazole is a prodrug that enters the cell by passive diffusion and is activated when its nitro group is reduced. Reduced metronidazole can interact with DNA causing strand breakage and helix destabilization, which leads to cell death (Odenholt *et al.*, 2007; O'Grady *et al.*, 2021). Vancomycin is a glycopeptide antimicrobial with bacteriostatic activity that inhibits peptidoglycan biosynthesis in the cell wall in gram-positive bacteria (Odenholt *et al.*, 2007; O'Grady *et al.*, 2021). In higher concentrations of vancomycin (8 and 16 mg/l), a bactericidal effect was observed (Odenholt *et al.*, 2007). Fidaxomicin is a narrow-spectrum macrocyclic antibiotic that targets bacterial RNA polymerase, with a bactericidal activity against Clostridia belonging to clusters I and XI and gram-positive nonspore-forming rods and anaerobic gram-positive cocci (Babakhani *et al.*, 2011; Finegold *et al.*, 2004). When the killing kinetics is compared, metronidazole exerted a very rapid bactericidal effect (<4 $\log_{10}$ colony-forming unit [CFU] after 3 hours) but in concentrations of 8 x minimum inhibitory concentration (MIC) (4 mg/l) and above (Odenholt *et al.*, 2007). Overall, vancomycin gave less kill than metronidazole (Odenholt *et al.*, 2007), and slower killing kinetics were also found in vancomycin than fidaxomicin. The bacterial count of *C. difficile* cells treated with vancomycin (4 × MIC) dropped slightly over two logs in 48 hours, whereas in the fidaxomicin experiment, the bacterial counts dropped below the detection limit (100 CFU ml-1) by 48 hours (Babakhani *et al.*, 2011).

Antimicrobial susceptibility testing (AST) recommendations differ between the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI); however, both bodies do not recommend using the broth microdilution method due to the difference in MICs compared with those yielded by agar dilution (Clinical and Laboratory Standards Institute, 2020; EUCAST, 2022; Hastey *et al.*, 2017). EUCAST recommends "fastidious anaerobe agar" for the AST of anaerobes, and the break points for *C. difficile* are based on epidemiological cutoff values; >2mg/l for metronidazole and vancomycin resistance. CLSI recommends "Brucella blood agar" supplemented with 5% sheep blood, hemin, and vitamin K1 for AST of anaerobes, with MIC break points for *C. difficile* of ≥32 mg/l and ≥4 mg/l for metronidazole and vancomycin, respectively. For metronidazole, it is important to note that very recent data shows that the consistent detection of metronidazole resistance is dependent on the presence of heme in agar media and its protection from light (Boekhoud *et al.*, 2021; Wu *et al.*, 2021). Both agars recommended by EUCAST and CLSI contain heme but in unknown quantities because of supplementation by blood. For fidaxomicin, there is still no official MIC break point available; 0.25 mg/l was suggested based on the MICs from European isolates (Freeman *et al.*, 2015).

Antimicrobial resistance in human *C. difficile* isolates showed equal-weighted pooled resistance for metronidazole and vancomycin of 1.0% (95% CI 0-3% and 0-2%, respectively) with a break point of >2 mg/l in a recent meta-analysis, including data for 5900 *C. difficile* isolates tested for metronidazole susceptibility and 11,188 *C. difficile* isolates tested for susceptibility to vancomycin (Sholeh *et al.*, 2020). When also analyzing *C. difficile* isolates from nonhuman sources, the weighted pooled resistance increased to 1.9% (95% CI 0.5-3.6%) for metronidazole and to 2.1% (95% CI 0-5.1%) for vancomycin (Saha *et al.*, 2019). For fidaxomicin, a few isolates with MICs from 1-64 mg/l were found recently in several studies investigating a large number of isolates (Freeman *et al.*, 2020; Goldstein *et al.*, 2011; Karlowsky *et al.*, 2020; Peng *et al.*, 2017; Schwanbeck et al., 2019); however, it should be noted that no commercial E-test for fidaxomicin is available in the market, so routine antimicrobial susceptibility data are limited.

MICs can differ according to *C. difficile* ribotype (RT). Elevated geometric mean MICs relative to other RTs were found in RTs 001, 027, 106, and 356 for metronidazole and in RTs 018 and 356 for vancomycin. These RTs belonged to epidemic types occurring in several European countries, except for RT356, which is probably genetically related to RT018 based on 94% similarity of polymerase chain reaction ribotyping banding profile, and was found only in Italy (Freeman *et al.*, 2015).

The clinical importance of MICs of metronidazole was highlighted in the study of Gonzales-Luna and colleagues. In the study cohort of 356 patients, increased MICs (≥1 µg/ml) have been identified as an independent predictor for clinical failure in patients with CDI treated with metronidazole (odds ratio 2.27; 95% CI 1.18-4.34); the majority of strains with a metronidazole MIC ≥1 µg/ml were RT027 (n = 45/65 [69%]) (Gonzales-Luna *et al.*, 2021).

The molecular mechanisms of resistance to metronidazole and vancomycin in *C. difficile* remain poorly understood. It is hypothesized that resistance to metronidazole is likely due to multifactorial processes involving alterations to metabolism with nitroreductases, iron uptake, active efflux, drug inactivation, DNA repair, or biofilm formation (O'Grady *et al.*, 2021). Vancomycin resistance mediated by *van* genes is very well described in *Enterococcus* sp. However, these gene clusters are also present in *C. difficile* but without corresponding vancomycin resistance phenotypes (O'Grady *et al.*, 2021). Recently, the presence of plasmid pCD-METRO and its international dissemination has been reported in both toxigenic and nontoxigenic *C. difficile* strains, with reduced susceptibility to metronidazole (Boekhoud *et al.*, 2021). Recently, plasmid-mediated resistance to vancomycin was also described in *C. difficile* isolate from a patient with CDI nonresponding to vancomycin treatment (Pu *et al.*, 2021). For fidaxomicin, several different mutations have been reported in laboratory-generated mutants, leading to alteration of fidaxomicin susceptibility (O'Grady *et al.*, 2021). An amino acid substitution V1143D in the RpoB was identified in clinical *C. difficile* isolate with MIC of >64 mg/l. This genetic change was also associated with reduced toxin A/B production and moderately reduced spore formation (Schwanbeck *et al.*, 2019).

It should be noted that fecal concentrations of vancomycin and fidaxomicin are many times greater than MICs detected in resis-

tant *C. difficile* isolates than metronidazole with low stool concentration (Bolton and Culshaw, 1986; Sears *et al.*, 2012; Thabit and Nicolau, 2015).

4.2. Clostridium difficile *spores*

Sporulation allows *C. difficile* to persist in the host and disseminate through environmental contamination. The persistence of *C. difficile* spores in the gut can play a role in the recurrence (relapse) of CDI (Chilton *et al.*, 2016). Significant inhibition of *C. difficile* sporulation *in vitro* was not observed with either metronidazole or vancomycin, but both fidaxomicin and OP-1118 inhibited sporulation, including for the epidemic NAP1/BI/027 strain (Babakhani *et al.*, 2012; Garneau *et al.*, 2014). Antimicrobial activity on *C. difficile* spores was detected in fidaxomicin-exposed spores but was absent in the vancomycin-exposed spores after washing in phosphate-buffered saline or in the more *in vivo* reflective fecal filtrate. The retention of antimicrobial activity prevented the recovery of spores on selective agar (Chilton *et al.*, 2016).

*5. Effect of the gut microbiota on the activity of anti-*C. difficile *infection agents*

It is unknown if the measured activities of antibiotics *in vitro* in a very well-defined environment against pure cultures of *C. difficile* represent what occurs *in vivo* in the intestinal tract with the presence of various other bacterial species and metabolites, which vary across individuals. Using *in vitro* models with bacterial communities, the activity spectrum of antibiotics, in general, is more complicated than testing *in vitro* with one species and one agent (Maier *et al.*, 2021). A further consideration is the effect of feces on the bioactivity of antibiotics used to treat CDI. The inactivation of metronidazole in the presence of gut contents was shown in the study of Rafii and colleagues, suggesting nitroreductase-producing enterococci as the possible cause (Rafii *et al.*, 2003). Importantly, a recent study using feces samples collected from 18 healthy individuals observed reduced antibiotic bioactivity of all three anti-CDI antimicrobials at 24 hours; however, the observed mean decreases for fidaxomicin (2.8-fold) or for vancomycin (1.5-fold) are unlikely to impact treatment efficacy due to the high fecal concentrations achieved. In contrast to vancomycin and fidaxomicin, a 727-fold reduction of bioactivity for metronidazole was seen and, considering the suboptimal stool concentration of this antibiotic, could be expected to have an impact on treatment outcome (personal communication with Mark Wilcox).

6. "Collateral damage" to the gut microbiota

Metronidazole, vancomycin, and fidaxomicin are bactericidal antibiotics with different mechanisms of action (Figure). The target site of antibiotics is one factor that determines the narrowness of the antibacterial spectrum. Fidaxomicin has very little effect or no activity against gram-negative aerobic and anaerobic bacteria, which likely contributes to the rapid recovery of the markedly disrupted microbiota found in patients with CDI (Louie *et al.*, 2012). Metronidazole affects the gut microbiome to a larger extent than vancomycin because it is active against anaerobic bacteria, including gram-negative anaerobes, primarily *Bacteroides, Fusobacterium,* and *Prevotella* spp., and also gram-positive anaerobes, such as *Peptostreptococcus* and *Clostridium* spp. Vancomycin inhibits various aerobic and anaerobic gram-positive bacteria, including other *Clostridium* spp. (Louie *et al.*, 2012). Although vancomycin is not normally active against gram-negative bacteria, vancomycin's very high intestinal concentrations can suppress the *Bacteroides*/*Prevotella* group bacteria (Louie *et al.*, 2012; Newton *et al.*, 2013). *Bacteroides* play an important role in colonization resistance due to their numerous presences on the mucosal surface and interference with intestinal pathogens (Eckburg *et al.*, 2005). In addition, vancomycin decreased fecal secondary bile acids, which inhibit the growth of the vegetative form of *C. difficile* (Vrieze *et al.*, 2014). Importantly, the changes in the gut microbiota and their metabolites (metabolomics) by vancomycin and metronidazole may persist for a considerable time period and affect various host functions, including immune regulation and metabolic activities (Soto *et al.*, 2018; Vrieze *et al.*, 2014).

7. Acquisition and overgrowth of vancomycin-resistant *Enterococci*

The changes in the recommendations for the treatment of CDI toward the use of vancomycin led to concerns of increased selective pressure for vancomycin-resistant enterococci (VRE). No increased risk for VRE acquisition has been identified in patients treated with metronidazole or vancomycin (Stevens *et al.*, 2020). Interestingly, a reduced acquisition of VRE (7% vs 31%, respectively; P <0.001) and Candida species (19% vs 29%, respectively; P-value = 0.03) was observed in patients who were treated with fidaxomicin versus those treated with vancomycin (Nerandzic *et al.*, 2012).

In addition, in patients with pre-existing VRE, a significant decrease in the mean concentration in stool was detected in the fidaxomicin group (5.9 vs 3.8 $\log_{10}$ VRE/g stool; P-value = 0.01) but not in the vancomycin group (5.3 vs 4.2 $\log_{10}$ VRE/g stool; P-value = 0.20), (Nerandzic *et al.*, 2012). In contrast, no significant difference in the density of VRE was observed after the onset of CDI therapy (during therapy or up to 2 weeks after completion of therapy, P >0.35) comparing vancomycin and metronidazole CDI treatment groups (Al-Nassir *et al.*, 2008).

8. *C. difficile* shedding

The treatment selection affects the clinical outcome of the patient but may also have an impact on the *C. difficile* shedding and environmental contamination and thus play a role in reducing health care-associated *C. difficile* transmission. Two prospective observational studies found fidaxomicin to be associated with lower rates of *C. difficile* contamination of the hospital environment than metronidazole and/or vancomycin. The study of Biswas and colleagues showed that patients treated with fidaxomicin (25/68, 36.8%) were less likely to contaminate their environment than patients treated with metronidazole and/or vancomycin (38/66 [57.6%], P-value = 0.02) (Biswas *et al.*, 2015). In the study of Davies and colleagues, observed rates of environmental contamination were 30% versus 50%, P-value = 0.04, on days 4-5 and 22% versus 49%, P-value = 0.005, on days 9-12 in five-room sites sampled of fidaxomicin or vancomycin/metronidazole recipients, respectively (Davies *et al.*, 2020). These data were further supported by the results of a prospective, unblinded, randomized, controlled trial, where contrary to observational studies, the metronidazole, vancomycin, and fidaxomicin treatment arms were evaluated separately (Turner *et al.*, 2022). Fidaxomicin and vancomycin were associated with a more rapid decline in *C. difficile* stool shedding than metronidazole (-0.36 $\log_{10}$ CFUs/d, -0.17 $\log_{10}$ CFUs/d, and -0.01 $\log_{10}$ CFUs/d, respectively. Both vancomycin and fidaxomicin (6.3% vs 13.1%) were associated with lower rates of environmental contamination than metronidazole (21.4%), respectively. With specific modeling of within-subject change over time, fidaxomicin was associated with a more rapid decline in environmental contamination than vancomycin or metronidazole (adjusted odds ratio, 0.83, 95% CI 0.70-0.99; P-value = 0.04), (Turner *et al.*, 2022).

9. Conclusion

The microbiological and pharmacological data support the CDI treatment recommendation of leaving metronidazole as the third alternative option only when fidaxomicin or vancomycin is not available or feasible. Oral vancomycin and fidaxomicin reach very high concentrations in the stool, but only fidaxomicin has a minimal effect on gut microbiota, inhibits sporulation, and shows antimicrobial activity on spores.

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Ethical approval

No ethical approval was necessary for this type of study.

Author contributions

M.K. conducted the literature search for this narrative review and wrote the original draft. E.J.K. and M.H.W. reviewed and commented on subsequent versions. All authors read and approved the submitted version.

Declaration of Competing Interest

M.K. and E.J.K. have no competing interests to declare. M.H.W. received lecture fees from Merck, Pfizer, and Seres and consultation fees from AiCuris, Bayer, Crestone, Da Volterra, Deinove, EnteroBiotix, The European Tissue Symposium, Ferring, GSK, Menarini, Merck, Nestlé, Paratek, Pfizer, Phico Therapeutics, Qpex Biopharma, Seres, Surface Skins, Summit, and Vaxxilon/Idorsia.

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