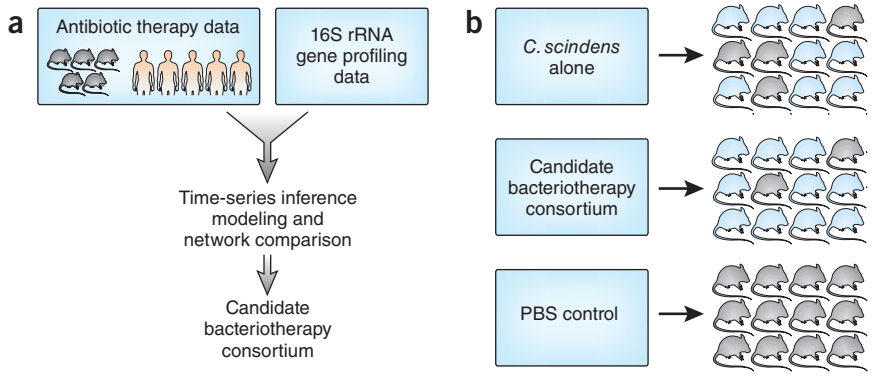

Figure 1 Systematic candidate bacteriotherapy selection. (**a**) Time-series inference modeling and 16S rRNA profiling enabled Buffie *et al.*[1] to identify bacteriotherapy candidates based on differential, antibiotic-induced changes in microbiome dynamics that resulted in susceptibility to *Clostridium difficile* infection, in both mouse models and a cohort of human patients. (**b**) *Clostridium scindens* and a consortium of three other bacteria that were found in both murine and human samples were used prophylactically to prevent infection by *C. difficile* after antibiotic treatment in a mouse model. Protected mice are colored blue, susceptible mice are colored gray. The consortium provided the best protection from *C. difficile*, but *C. scindens* alone also inhibited *C. difficile*.

understanding needed for unraveling how such therapies might work.

Buffie *et al.*[1] used an elegant screening strategy that was based on differential susceptibility of mice to *C. difficile* infection after pretreatment with three different antibiotics, each of which differ in their effects on the microbiota. Clindamycin induced robust, extended susceptibility to infection, ampicillin induced transient susceptibility to infection and enrofloxacin induced no susceptibility to infection. The authors applied multiparallel 16S rRNA gene profiling and inference modeling of time-series data to analyze variations among the gut microbiota upon treatment with the three antibiotics (**Fig. 1a**). This unbiased approach identified 11 bacterial operational taxonomic units that were statistically correlated with resistance of antibiotic-treated mice to *C. difficile*.

They then examined the microbiota of a cohort of allogeneic hematopoietic stem-cell transplant patients (who had received prophylactic antibiotic treatment), including 12 who developed *C. difficile* disease and 12 who were asymptomatic carriers, using a systems biology approach. Computational integration using inference modeling of antibiotic schedules with time-resolved multiparallel 16S rRNA gene profiling of the microbiota from these samples produced networks suitable for comparison with the mouse data. This comparison revealed that two bacterial species that resisted *C. difficile* infection were conserved between humans and mice, with one of them, *C. scindens*, being most highly correlated with resistance.

Next, *C. scindens* was administered alone, or together with three cultivated, resistance-associated species, in a mouse model of *C. difficile* disease. Mice were treated with antibiotics, then with therapeutic bacteria, and finally infected with *C. difficile* (**Fig. 1b**). Both therapies increased resistance to infection, reducing the *C. difficile* population more than 100-fold, and significantly improving survival. Although the consortium provided better protection than *C. scindens* alone, the latter was the only species individually capable of providing protection (**Fig. 1b**).

To understand how *C. scindens* alone could control *C. difficile* disease, Buffie *et al.*[1] undertook a mechanistic investigation, correlating relative abundance of secondary bile acid and bile acid–inducible (bai) genes with resistance to infection. They observed that treatment with either *C. scindens* or the four-member consortium increased the relative abundance of the secondary bile-acid products lithocholate and deoxycholate, which inhibited *C. difficile* growth in a concentration-dependent manner *in vitro*. The ability to metabolize bile to these products is most prevalent among clostridia; however, in the consortium, only *C. scindens* harbors *baiCD* genes. It is not known whether *baiCD* or other genes encoding bile metabolism are present in the Lachnospiraceae D4 isolate identified by Reeves *et al.*[7]. Resistance to colonization by *C. difficile* has previously been associated with the specific metabolite state of the host[8], which might indicate that infection is dependent on bile acid, a finding that could be exploited therapeutically with further research. In support of this hypothesis, the most well-characterized *C. difficile* germination factor, taurocholate, is a primary bile acid[9].

The greater efficacy, reduced weight loss and lower infection rate conferred by the consortium compared with *C. scindens* alone suggest that the consortium has synergistic mechanisms of action, which might include immunomodulation or competition for resources and metabolites. As any human bacteriotherapy should resolve clinical symptoms and, ideally, provide complete resistance to infection, these results imply it will likely be necessary to combine several species into a single therapeutic. Like other forms of combination therapy, a consortium based on diverse mechanisms of protective action might also reduce the emergence of *C. difficile* resistance compared to a single-species probiotic. Such an approach would require mechanistic understanding of multiple candidate therapeutics, as Buffie *et al.*[1] have begun to achieve with their work.

The next phase of this research will involve rational selection of further bacteriotherapy candidates capable of suppressing infection and symptoms in animals to a level acceptable for clinical translation. Buffie *et al.*[1] used 16S rRNA

(continued)

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