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# Targeting the Gut Microbiome as a Treatment for Primary Sclerosing Cholangitis: A Conceptual Framework

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**Primary sclerosing cholangitis (PSC) is a rare, immune-mediated, chronic cholestatic liver disease associated with a unique phenotype of inflammatory bowel disease that frequently manifests as pancolitis with right-sided predominance. Available data suggest a bidirectional interplay of the gut-liver axis with critical roles for the gastrointestinal microbiome and circulating bile acids (BAs) in the pathophysiology of PSC. BAs shape the gut microbiome, whereas gut microbes have the potential to alter BAs, and there are emerging data that alterations of BAs and the microbiome are not simply a consequence but the cause of PSC. Clustering of PSC in families may suggest that PSC occurs in genetically susceptible individuals. After exposure to an environmental trigger (e.g., microbial byproducts or BAs), an aberrant or exaggerated cholangiocyte-induced immune cascade occurs, ultimately leading to bile duct damage and progressive fibrosis. The pathophysiology can be conceptualized as a triad of (1) gut dysbiosis, (2) altered BA metabolism, and (3) immune-mediated biliary injury. Immune activation seems to be central to the disease process, but immunosuppression does not improve clinical outcomes or alter the natural history of PSC. Currently, orthotopic liver transplantation is the only established life-saving treatment, whereas antimicrobial therapy or fecal transplantation is an emerging therapeutic option for PSC. The beneficial effects of these microbiome-based therapies are likely mediated by a shift of the gut microbiome with favorable effects on BA metabolism. In the future, personalized approaches will allow to better target the interdependence between microbiome, immune function, and BA metabolism and potentially cure patients with PSC.**

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## INTRODUCTION

### Definitions and clinical presentation

Primary sclerosing cholangitis (PSC) is an uncommon chronic cholestatic liver disease characterized by inflammation and fibrosis of intra- and extrahepatic bile ducts, leading to the formation of multifocal bile duct strictures and progressive fibrotic transformation of bile ducts (1). Hepatic fibrosis is promoted by these biliary changes, ultimately leading to cirrhosis and liver failure (2). There is a strong association between PSC and inflammatory bowel disease (IBD), particularly ulcerative colitis (UC). Between 2.5% and 7.5% of individuals with IBD will eventually develop PSC. Conversely, between 60% and 70% of patients with PSC have IBD (3). Furthermore, the phenotype of IBD occurring in PSC is unique and differs from the typical phenotype seen in patients with UC or Crohn's disease (CD) (4). Patients with PSC-IBD typically have mild intestinal disease activity and an increased incidence of extensive colitis typically pancolitis, with a right-sided predominance, rectal sparing, and backwash ileitis.

### Diagnosis

In the clinical setting, a diagnosis of PSC (1) is made in patients with a cholestatic biochemical profile when cholangiography (e.g., magnetic resonance cholangiography or endoscopic retrograde

cholangiography) shows characteristic bile duct changes with multifocal strictures and segmental dilatations, and secondary causes of sclerosing cholangitis have been excluded (5).

### Epidemiology

PSC occurs more commonly in men than in women (2:1). The mean age at the time of diagnosis is approximately 40 years (6). Using a random-effects model, a systematic review and meta-analysis concluded that the pooled incidence rate for PSC was 0.77 (0.45–1.09) per 100,000 person-years (7).

### Morbidity and mortality

PSC is often progressive, leading to fibrosis, cirrhosis, and end-stage liver disease. In the absence of any pharmacological therapy, orthotopic liver transplantation (OLT) represents the only curative option (8,9). The median life expectancy after a diagnosis of PSC is 13.2–21.3 years without liver transplantation (6). There is a 3-fold mortality rate increase (hazard ratio 2.92, 95% CI 2.16–3.94) and a 2-fold increase in risk of any malignancy (hazard ratio 2.23, 95% CI 0.88–6.11) in patients with PSC compared with the general population; (10). However, cancer-related death far exceeds death caused by end-stage liver disease and other non-malignant complications of the disease (11).

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## PATHOPHYSIOLOGY

The pathophysiology of PSC is multifactorial, with genetic and environmental factors implicated.

### Genetics

It is now believed that PSC occurs in genetically susceptible individuals (12) after exposure to an environmental trigger (13). This exposure initiates a series of events involving complex interactions between the innate and adaptive immune systems, ultimately leading to gut-derived activated lymphocytes migrate to the liver, cholangiocyte damage, and progressive fibrosis (8). The obvious association between PSC and IBD points toward a possible role of autoimmunity (2). Indeed, characteristic human leukocyte antigen haplotype associations have been recognized in PSC (2), and large-scale genome-wide association studies have identified genotypic associations in cohorts with PSC (14,15). More than 70% of patients with PSC also have IBD, and at least 2 common gene loci have been identified in genome-wide association studies for PSC and UC (14). Overall, the most plausible explanation for the pathogenesis of PSC is immunogenic priming in a genetically predisposed individual, leading to the characteristic phenotype, that is likely further influenced by other host or environmental factor (8).

### Host/patient immune response

Several important observations, coupled with the strong association between certain human leukocyte antigen haplotypes and frequency of concurrent extrahepatic autoimmune disorders, support the concept that PSC is an immune-mediated phenomenon. The possible role of liver-gut crosstalk in PSC and UC pathogenesis may be related to the enterohepatic circulation of lymphocytes (16) referred to as the gut lymphocyte homing hypothesis. This theory postulates that a subset activated T cells from the gut appear home in on the liver, then initiate and inflict damage (17).

### Gastrointestinal microbiota

As more than 60% of patients with PSC have coexistent IBD, the pathogenesis of PSC seems to be related to the manifestation of the inflammatory changes of the bowel that seem to mirror UC. It is now widely accepted that the microbiome plays a key role for the manifestation and progression of IBD (18). Similarly, there is now accumulating evidence that alterations of the gastrointestinal microbiome are central to the pathogenesis of PSC (19). Several clinical trials (Table 1) provide evidence that the stool microbiome and the microbiome adherent to the colonic mucosa are distinct in patients with PSC compared with patients with IBD without PSC and non-IBD controls.

The thus far largest study on the microbiome (20) included 137 patients with PSC and found differences in the microbiota profiles between patients with PSC and UC. Despite the observed dysbiosis, it was not possible to differentiate PSC and UC based on microbial taxonomic characteristics. The taxonomic differences between patients with PSC and UC may have pathophysiological significances (21). Microbes can contribute—either in isolation or collectively via specific metabolic products and/or interference with the mucosal immune system—toward the pathophysiology of PSC (or IBD). Several studies (20,22–27) have shown that patients with PSC have an overrepresentation of *Veillonella* spp. This taxon (and others enriched in patients with PSC-IBD) can produce amine

oxidases. Amine oxidase such as vascular adhesion protein-1 (VAP-1) facilitates adhesion of gut-tropic lymphocytes to the liver endothelium (28) and potentially contributes to the manifestation of PSC-IBD via aberrant lymphocyte tracking between the bowel and liver (29). The alteration in the relative abundance of *Veillonella* in the gut microbiome is not specific for PSC and is potentially a marker of advanced liver disease (30,31). In addition, Vieira-Silva et al. (32) have very observed that an increase in *Enterococcus* spp. is positively associated with serum alkaline phosphatase (ALP) levels and bile duct obstruction. They also reported that increases in *Veillonella* and *Fusobacterium* spp. are both capable of driving the proinflammatory burden associated with PSC and PSC-IBD but are coexclusive, with members of the latter genus favored in patients with CD or PSC-CD. Lemoine et al. (25), reported fungal dysbiosis in patients with PSC. They found a decrease in the proportion of the Saccharomycetaceae family, including the species *Saccharomyces cerevisiae*, known to have anti-inflammatory properties and shown to be decreased in patients with active IBD (33). All these findings suggest that the gut microbiome might be involved in the pathogenesis of PSC (and associated IBD) while patients with PSC and IBD have a microbiome that is distinct from patients with IBD alone. Thus far, no specific microbial signature unique to PSC has been identified based on relative abundances alone, and more studies with comprehensive functional and quantitative characterization of the microbiome are warranted.

### Interplay between gastrointestinal microbiome, BA metabolism, and immune function in PSC

**The gastrointestinal microbiome and metabolism of BAs.** The human gut microbiota consists of thousands of different bacterial species and other microorganisms with defined functions. This microbial ecosystem maintains homeostasis through a tight balance between the mucosal immune system, cell-to-cell signaling, and subsequent release of antimicrobial peptides to control neighboring bacterial clades (34). Commensal microbial metabolism produces essential vitamins and short-chain fatty acids (SCFAs) and modifies a variety of endogenous and exogenous sources of complex molecules (e.g., BAs and xenobiotics) that can contribute to the gut metabolome (35,36). The SCFAs, particularly butyrate, regulate innate and adaptive immune cell generation, trafficking, and function and have anti-inflammatory effects. Thus, SCFAs play an active role in modulating the mucosal immune system to establish a tolerant phenotype against beneficial commensal microbiota (37). However, the biodiversity of gut microbes implicated in coordinating BA metabolism and their conversion(s) from primary to secondary BAs is narrow and perhaps better defined from a genetic and biochemical context than other aspects of microbial metabolism in the human gut (38).

Hepatocytes synthesize primary BAs, cholic acid (CA), and chenodeoxycholic acid from cholesterol, which are conjugated with taurine or glycine (making them more water soluble) for secretion into bile. Primary BAs are secreted into the bile ducts and are stored in the gallbladder during the interdigestive phase (39). After a meal, cholecystokinin (CCK) released from the pancreas stimulates gallbladder contraction to release BAs into the duodenum. A small proportion of bile secreted into the duodenum reaches the terminal ileum and colon (40), where 95% of BAs are absorbed by active transport and transported via the portal vein to the liver where they are taken up by hepatocytes and re-excreted. This process is known as enterohepatic circulation. Microbes in the ileum and colon transform primary BAs (CA and

**Table 1. Studies of the gut microbiota in PSC**

No.	Study	Year	Material	PSC, n	Diversity	Specific changes in the gut microbiome in PSC, using 16S rRNA gene sequencing
1	Rossen et al. (92)	2015	Ileocecal mucosa	12	Reduced	Clostridiales↓
2	Kevans et al. (26)	2016	Colon mucosa	31	No change	No consistent indicator found
3	Torres et al. (93)	2016	Terminal ileum, right and left colon mucosa	20	No change	Barnesiellaceae and <i>Blautia</i> ↑
4	Kummen et al. (22)	2016	Stool	85	Reduced	<i>Veillonella</i> ↑
5	Quaraiishi et al. (29)	2016	Colon mucosa	11	NA	<i>Escherichia</i> , Lachnospiraceae, and <i>Megasphaera</i> ↑
6	Sabino et al. (27)	2016	Stool	52	Reduced	<i>Enterococcus</i> , <i>Lactobacillus</i> , and <i>Fusobacterium</i> ↑
7	Ruhlemann et al. (23)	2016	Stool	73	NA	<i>Veillonella</i> ↑
8	Bajer et al. (24)	2017	Stool	43	Reduced	<i>Rothia</i> , <i>Enterococcus</i> , <i>Streptococcus</i> , <i>Clostridium</i> , <i>Haemophilus</i> , and <i>Veillonella</i> ↑ <i>Coprococcus</i> , <i>Prevotella copri</i> , <i>Ruminococcus gnavus</i> , <i>Faecalibacterium prausnitzii</i> , and <i>Adlercreutzia</i> ↓
9	Vaughn et al. (44) <sup>a</sup>	2019	Stool	7	Reduced	<i>Bacteroides</i> ↓ and <i>Megamonas</i> ↑
10	Ruhlemann et al. (20)	2019	Stool	137	Reduced	Proteobacteria↑ and <i>Parabacteroides</i> ↑
11	Lemoine et al. (25)	2019	Stool	49	Reduced	Fungal dysbiosis: <i>Exophiala</i> ↑ and <i>Saccharomyces cerevisiae</i> ↓ Bacterial dysbiosis: Firmicutes↓ (except for <i>Veillonella</i> ↑) and Proteobacteria↑

Six patients with PSC/IBD were administered a 2-week course of oral vancomycin, 500 mg twice a day, with no significant improvement in cholestatic liver enzymes or indicators of disease activity. Oral vancomycin administration substantially decreased alpha diversity and secondary bile acid metabolism, both of which recovered within 3 weeks of vancomycin cessation.

<sup>a</sup>No substantial differences were found in the fecal bile acid profiles of patients with IBD and PSC (n = 7) compared with IBD alone (n = 8) or healthy controls (n = 8). IBD, inflammatory bowel disease; NA, not applicable; PSC, primary sclerosing cholangitis.

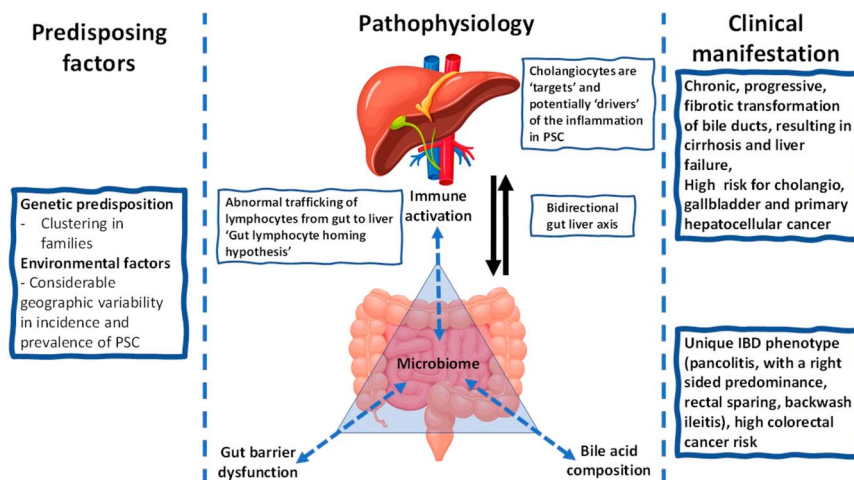
chenodeoxycholic acid) into hydrophobic secondary BAs (lithocholic acid and deoxycholic acid) by wide array of bacterial transformation including deconjugation catalyzed by the bile salt hydrolase, dehydroxylation by 7- $\alpha$  dehydroxylase, oxidation/reduction, and epimerization (41). Some of these secondary BAs are reabsorbed during colonic transit and are also circulated in portal blood to the liver. The enterohepatic circulation of the highly hydrophobic and toxic secondary BAs and increased concentrations in the liver have been linked to inflammation, cholestasis, gallstone formation (42), and carcinogenesis (43). Because of the colonic absorption of BAs, only a small proportion of BAs (0.5 g/d) is excreted in the feces. These are replaced by *de novo* synthesis of BAs in the liver. This could explain the similarity of fecal BA profiles in patients with IBD and PSC, IBD alone, or healthy controls (44).

There is emerging evidence that luminal bile acids affect the gastrointestinal microbiome. BAs have antimicrobial activity by damaging the bacterial cell membrane and thus inhibiting bacterial outgrowth (45). Reduced BA concentrations are associated with bacterial overgrowth (46,47). Indeed, a recent systematic review and meta-analysis (48) suggested that advanced chronic liver disease is associated with bacterial overgrowth. Gut bacteria metabolize BAs and thus influence BA composition and BA hydrophobicity (49). These BAs also shape the gut microbiota communities, whereas intestinal microbes alter BAs. Thus, there is a bidirectional interaction

between the intestinal microbiota and BAs (50). Thus, there is a link between gastrointestinal microbes and hepatic BA metabolisms. This gut-to-liver axis may play a critical role in the regulation of enterohepatic circulation of BAs, BA pool size, and BA composition. Alterations of the BA homeostasis and excessive intrahepatic accumulation of (potentially toxic) BAs and/or their metabolites are thought to play a pivotal role in mediating the hepatic injury of cholestatic diseases (51).

**BA metabolism and mucosal immune function.** The regulatory functions of BAs are mainly the result of activation of intracellular ligand-activated nuclear receptors, such as the farnesoid X receptor (FXR, NR1H4) and cell surface G protein-coupled receptors, specifically the G protein-coupled BA receptor (TGR5 or GPBAR1) (52). Both receptors are highly represented in cells of innate immunity such as intestinal and liver macrophages, dendritic cells, and natural killer T cells. Thus, both FXR and TGR5 are critical regulators of BA metabolism, BA circulation, and intestinal immune function and contribute to the maintenance of a tolerogenic phenotype in enterohepatic tissues (52).

Bile acids also directly shape mucosal immune function via interactions with both dedicated BA receptors (FXR and GPBAR1) and nonspecific BA sensors (pregnane X receptor, vitamin D receptor, and constitutive androstane receptor). In the context of innate immunity (53–55), BAs suppress NF- $\kappa$ B-dependent signaling pathways (56) and inhibit NLRP3-dependent inflammasome



**Figure 1.** Conceptual framework for primary sclerosing cholangitis (PSC) exploring the interrelationship between bidirectional gut-liver axis.

activities (55). A recent study by Hang et al. (57) showed that BAs directly regulate the adaptive immune cells via proinflammatory Th17 cells and anti-inflammatory regulatory T cells (Tregs). A successful host immune response is generally the result of both pro- and anti-inflammatory elements that are carefully tuned with the goal of clearing the pathogen while limiting host damage (autoimmunity) (58). Thus, gut microbes can modify BAs, which in turn regulate the gut immunity and inflammation. Furthermore, the impact of mucosal immune function on BA metabolism is currently unknown and will be an area of future investigation. The 3-dimensional interplay between BAs, the microbiota, and the mucosal immune system, proposed by Chen et al. (59), likely plays a crucial role in regulating the mucosal immune homeostasis.

**Gut barrier function.** Although alterations of gut barrier function might be related to the mucosa-associated microbiome in the small intestine (60,61), there is also evidence that in patients with PSC, gut barrier dysfunction is associated with a poor outcome (62). Bacterial translocation and/or absorption of bacterial endotoxins such as lipopolysaccharide, lipoteichoic acid, and bacterial DNA/RNA fragments into the portal circulation via a chronically inflamed leaky bowel, combined with Kupffer cell activation, may be crucial in eliciting the proinflammatory, profibrotic hepatobiliary responses that lead to the development of PSC (5,63).

#### Gut barrier function and host-microbe interactions

The host-microbe interactions may play a pivotal role for the so-called leaky gut and PSC microbiota hypotheses. Indeed, in an animal model of PSC, the epithelial-damaging effect of *Klebsiella pneumoniae* isolated from patients with PSC was linked to bacterial translocation and increased susceptibility to TH17-mediated injuries of bile ducts (64). These findings are well aligned with the concept that microbes or microbial products may lead to an aberrant or exaggerated immune response of the cholangiocyte (increased induction of cholangiocyte senescence and senescence-associated secretory phenotype), which plays a central role in the pathogenesis of PSC (5). This is further supported by the thought that the epithelial cells lining the bile ducts are not only a target of injury in PSC but also actively involved drivers in the course of disease (65,66).

This suggests an interrelationship between gastrointestinal microbes, BAs, and immune-mediated damage to the epithelial

lining of the bile ducts, results in an ongoing inflammatory process that defines the pathophysiology of PSC (Figure 1).

#### TREATMENT

A recent Clinical Guideline of the American College of Gastroenterology (67) concludes that there is “...no approved or proven therapy...” for PSC. On the other hand, there is some evidence that antibiotic therapy is beneficial in patients with PSC. Since an initial case series of a patient with PSC was published in 1958 (68), a number of treatments including immune suppression have been tested, but none have proven to alter the course of disease or cure this disease. Indeed, until now, PSC has a poor prognosis, and OLT is so far the only proven therapy to extend life expectancy.

#### Treatment targeting the microbiome

Against the background of the American College of Gastroenterology clinical guideline, there is emerging evidence, albeit from relatively small open-label studies that in patients with PSC, targeted modulation of the gastrointestinal microbiome may alter the course of disease to delay or even stop disease progression. The first study on the potential effect of antibiotics in PSC was published in 1959 by Rankin et al. (69). Subsequent case series and studies in adults (70–74) testing various antimicrobial agents in the treatment of PSC demonstrated improvement in ALP and the Mayo PSC Risk Score as cohort markers for the progression of PSC (75,76). In a recent cohort study (77), 17 children were treated with oral vancomycin for UC related to PSC or autoimmune sclerosing cholangitis. In 15 of 17 patients, oral vancomycin therapy achieved mucosal healing (Mayo endoscopic score 0), with 9 achieving histological remission. LFTs improved transiently or normalized in at least 12 patients.

Our recent systematic review and meta-analysis (78) of available studies assessed and compared the efficacy of various antibiotics in adult patients with PSC with or without coexisting IBD with regard to (i) improvement of liver enzymes, (ii) Mayo PSC Risk Score, and (iii) number of adverse drug reactions resulting in discontinuation of antibiotic therapy. In this review, vancomycin seemed to be the most effective antibiotic with regard to clinical improvement and adverse effects. Most recently, a small case series by Dao A et al. (79) found that oral

vancomycin was effective for the induction and maintenance of remission of UC in adults with UC-PSC. All had long-standing disease (average duration 15 years), with 5 of 8 patients having undergone OLT for PSC. However, very little is known about the specific mechanisms, and alterations of the gastrointestinal microbiome are associated with response during treatment and relapse after discontinuation of therapy.

Overall, alterations of the gastrointestinal microbiome by antibiotic therapy seem to alter the natural course of PSC and delay the progression of disease. It might even be speculated that favorable permanent changes of the gastrointestinal microbiome could potentially cure PSC.

### Evolving disease concepts

PSC is frequently accompanied by IBD. However, the clinical features of IBD in patients with PSC are unique and distinct from typical patients with IBD (Table 2).

PSC is a progressive chronic inflammatory disease with fibro-obliterative destruction of extra- and intrahepatic bile ducts. These histologic features suggest that PSC is an immune-mediated disorder. However, immune suppression with steroids or other immunosuppressants does not seem to alter the natural history of PSC (80). Thus, other causes for the inflammatory process might be considered. Indeed, a recent systematic review and meta-analysis found that antibiotic therapy and in particular treatment with the nonabsorbable antibiotic vancomycin was associated with clinical improvement. The clinical and biochemical response was better for vancomycin compared with the other antibiotics studied (78). The fact that an antibiotic improved outcomes may further suggest that microbes are involved in the pathophysiology of PSC. Moreover, nonabsorbable antibiotics such as vancomycin were effective, likely suggesting that a gastrointestinal microbe is central to pathogenesis of PSC. Many Gram-positive bacteria are able to deconjugate, dehydroxylate, oxidize, and epimerize primary BAs into secondary BAs (81,82), whereas no deconjugation activity has been detected in Gram-negative intestinal bacteria, with the exception of 2 species of *Bacteroides* (83). Unlike deconjugation, dehydroxylation is only performed by a minor population of Gram-positive anaerobic *Clostridium* species (84).

Treatment with vancomycin specifically targets Gram-positive bacteria (85), which includes various *Clostridium* spp. known to be primarily involved with the dehydroxylation of primary BAs, into the secondary BAs present in the distal small intestine and colon (82,84). This relatively narrow specificity of vancomycin might be a potential explanation for its superior effects on PSC, whereas the other antibiotics (such as rifaximin, metronidazole, and minocycline) trialed in the treatment of PSC are widely considered to be broad spectrum. Thus, it is possible that vancomycin through its effect on gut microbes influences BA metabolism. In support of a selective effect of vancomycin on Gram-positive species, a recent RCT (85) of 20 males with metabolic syndrome showed that treatment with vancomycin—compared with amoxicillin—reduced fecal microbial diversity with a decrease in Gram-positive bacteria (mainly Firmicutes phylum, e.g., species belonging to the *Clostridium* clusters IV and XIVa) and a compensatory increase in Gram-negative bacteria (mainly Proteobacteria). Treatment with vancomycin not only decreased the fecal secondary BAs and increased the primary BAs but also decreased postprandial plasma concentrations of secondary bile salts and

increased that of primary bile salts. Vancomycin induced reduction of the hydrophobic secondary BAs, which are potentially responsible for the right-sided colitis and after absorption for mediating the bile duct injury, could perhaps explain its beneficial role in PSC.

In a mouse model (86), vancomycin was able to reverse mycophenolate mofetil (MMF)-induced GI toxicity (colonic inflammation and weight loss) by potentially restoring the MMF-induced dysbiosis of the microbiota. MMF alters the composition of the gut microbiota, selecting for bacteria expressing the enzyme  $\beta$ -glucuronidase (GUS) and leading to an upregulation of GUS activity, thereby leading to increased concentrations of active mycophenolic acid, which is associated with colonic inflammation. Oral vancomycin depleted classes Bacteroidia and Clostridia, while allowing expansion of Gammaproteobacteria and Bacilli. Although vancomycin has a relatively narrow antibiotic spectrum, it is likely that very specific bacteria are responsible for this anti-inflammatory effect.

Vancomycin, in addition to its effect on the gut microbes, perhaps also has an immunomodulatory effect. A study of 14 children with PSC and UC treated with oral vancomycin (87) showed that in addition to symptom resolution and normalization of liver biochemistry, there was an increase in peripheral CD4<sup>+</sup>FoxP3<sup>+</sup> regulatory T (Treg) cells and transforming growth factor beta (TGF- $\beta$ ) levels in response to the antibiotic, suggesting an immunomodulatory effect of vancomycin. The immunomodulatory effect of vancomycin was thought to be due to its direct effect on the T-cell inflammatory pathway via the TNF- $\alpha$  pathways and downstream Treg induction. This may suggest a immunomodulatory effect of vancomycin via the TNF- $\alpha$  inflammatory pathways and/or downstream Treg induction.

Fecal microbiota transplantation (FMT) refers to the transfer of intestinal microbes from a healthy donor into a recipient with the intent of modifying the recipient's intestinal microbiome. The pilot study (88) of 10 patients with PSC with concomitant IBD in remission who underwent FMT (from a single donor) showed  $\geq 50\%$  reduction in ALP levels in 30% of the treated patients with PSC. This study suggests that FMT treatment is safe and results in an increased stool microbial diversity that persisted after FMT. Engrafting OTUs included genera capable of producing short-chain fatty acids, which are known to be depleted in IBD. Larger controlled studies are needed to define efficacy and define the mechanisms of FMT in PSC. Figure 2 summarizes microbiome-based therapy, targeting key pathophysiologic mechanisms central to PSC. Although FMT increases the overall stool microbial diversity, oral vancomycin therapy is associated with reduced fecal microbial diversity. Thus far, more data point toward improved clinical outcomes in response to antibiotic therapy with vancomycin; both interventions might be effective, and the precise mechanisms of these microbiome-based therapies remain to be explored.

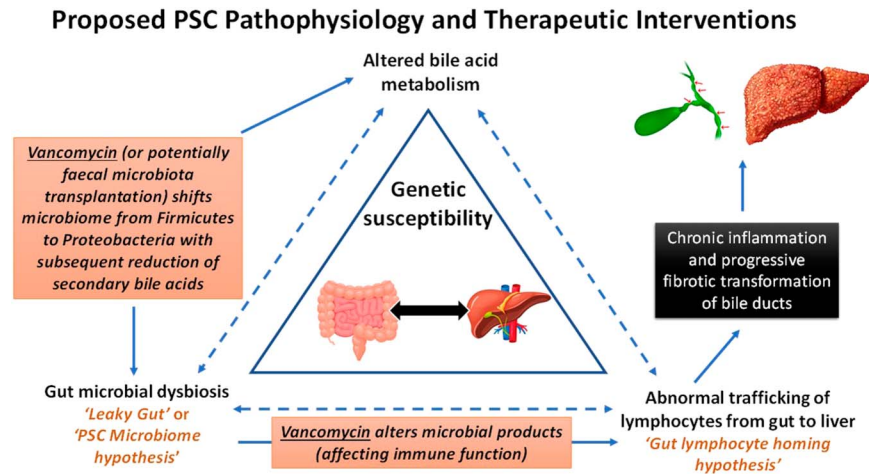
### FUTURE DIRECTIONS

Antimicrobial treatments targeting the gastrointestinal microbiome seem to result in significant improvement in the clinical and cholestatic biomarkers (78) and—based on case reports (87,89–91)—even long-term outcomes in PSC. FMT in PSC seems to be safe and is likely associated with a sustained improvement in the overall microbial diversity and liver enzymes. This points toward the gastrointestinal microbiome as an important disease modifier for PSC. So far, most microbiome research in PSC has focused on the stool

**Table 2. Challenges for the traditional concepts related to PSC and associated IBD**

Established concepts related to PSC in IBD (UC and CD)	Supporting or negating evidence
PSC is an immune-mediated disease	Immunosuppression has no effect on the clinical outcomes or on the natural history of PSC (20,80).
Treatment	
In patients with IBD without infective complications, antibiotic therapy is not a routine treatment (only exception patients with IBD with pouchitis)	Treatment with antibiotics improves clinical outcomes in PSC (78).
Faecal microbial transplantation improves UC in a substantial proportion of patients (94).	Fecal transplantation seems to be safe. Although only a small proportion of patients had improvement in ALP, bacterial diversity was increased in all patients as early as week 1, with persistence through to week 24, after a single FMT (88).
Probiotics improves IBD, particularly pouchitis <sup>95</sup> .	Probiotics seem to have no beneficial outcomes in patients with PSC with associated IBD (97,98).
Curcumin in combination with mesalazine induces remission in patients with mild to moderate UC (96).	Treatment with curcumin 750 mg orally twice daily for 12 weeks was not associated with significant improvements in cholestasis or symptoms in patients with PSC (99).
Ulcerative colitis is frequently associated with PSC	Phenotype of IBD in PSC is unique and different from IBD without PSC
	Unique characteristics of PSC associated with UC
	Predominantly right-sided colitis (100) and rectal sparing with backwash ileitis (8).
	After colectomy, patients with UC and PSC have an increased risk of acute and chronic pouchitis (101) development and bleeding of peristomal varices (102) compared with patients with UC.
	Risk of CRC in patients with PSC with UC
	Patients with PSC-UC have a 4-fold increased risk of CRC compared with UC alone (103) and 10-fold higher compared with the general population (104).
	Colorectal cancers in patients with PSC predominantly occur in the right colon (100).
Crohn's disease is associated with PSC	CD associated with PSC shows
	Predominantly colonic involvement in CD-PSC.
	Milder disease activity and reduced rates of penetrating and stricturing disease are lower (105).
	Risk of CRC in PSC and CD
	Like in PSC-UC, the CRC risk is substantially higher in patients with PSC-CD compared with CD alone.
Colectomy (a cure for UC) should improve PSC	Inconsistent results on the effect of colectomy on PSC
	Colectomy seems to have little effect on the progression of PSC and survival of patients (106).
	Some studies suggest pre-/peri-LT colectomy in patients with UC and PSC may have a protective role against recurrence of PSC (107)
OLT improves IBD in patients with PSC	Course of IBD after OLT for PSC is variable
	After OLT in PSC, approximately one-third of patients with known IBD improve, and one-third worsens (108).
	One study reported increased risk of colectomy for intractable IBD (109) after OLT, whereas other reported a reduced need for colectomy in patients with UC (110).
	Immunosuppressive agents used for OLT might affect IBD as well. Dual treatment with tacrolimus and mycophenolate mofetil was associated with increased IBD activity, and combination treatment with cyclosporine and azathioprine had protective effects (109).
	<i>De novo</i> manifestation of IBD can occur in patients with PSC after OLT despite immunosuppressive therapy (111).

ALP, alkaline phosphatase; CD, Crohn's disease; IBD, inflammatory bowel disease; FMT, fecal microbiota transplantation; OLT, orthotopic liver transplantation; PSC, primary sclerosing cholangitis; UC, ulcerative colitis.



**Figure 2.** Pathophysiology of PSC. Against the background of a genetic susceptibility, specific components of microbiome or metabolic products can trigger an exaggerated immune response from cholangiocytes. Alternatively (or in addition), the gut dysbiosis is responsible for altered metabolic function of the gut microbiome with subsequent changes of the bile acid pool or an increased intestinal permeability. Treatment with vancomycin (or potentially fecal microbiota transplantation) shifts the microbiome toward, e.g., Proteobacteria with subsequent effects on the production of secondary bile acids. This affects the bile acid pool and influences favorably cholangiocyte biology. PSC, primary sclerosing cholangitis.

microbiome, whereas some studies assessed the colonic mucosa-associated microbiome (Table 1). The bidirectional gut-liver axis makes it equally possible that the mucosa-associated microbiome in the small intestine harbors the microbes that are critical for the pathophysiologic process that causes PSC and the associated IBD. So far, there is cumulating evidence that the stool microbiome and the intestinal microbiome are different in patients with PSC compared with controls (Table 1), and this may explain the altered metabolic functions (e.g., in relation to metabolism of BAs) or immune function that are related to the pathophysiology of PSC.

Although the available data suggest that targeting the gut microbial dysbiosis either by antibiotic therapy (in particular vancomycin) or FMT might be an effective approach in at least a subgroup of patients with PSC, prospective randomized placebo-controlled trials are lacking. These trials also should explore the links between sustained alterations of the gastrointestinal microbiome during treatment and long-term outcomes. These data would be critical to better understand the precise mechanisms by which antibiotic therapy in PSC alters the clinical course and could potentially allow to develop nonantibiotic interventions that ultimately could cure with this currently fatal disease. Understanding the interdependence between microbiome, immune function, BA metabolism, and the changes induced by microbiome-targeting therapies such as antibiotics or FMT will allow us to define new targets for therapy and potentially develop a cure for patients with PSC. The ultimate solution might not necessarily be a treatment that targets the microbiome but a treatment that blocks either the metabolism of a microbial product or the effects of a microbial product(s) that drives the manifestation and progression of PSC.

There is a need for further in-depth studies using system biology approaches in combination with longitudinal multilevel analyses in patients with PSC (including appropriate controls with and without other cholestatic liver diseases) to elucidate potential targets for interventions. This ultimately will result in a better understanding of the unique clinical pattern of PSC and the factors that drive manifestation and disease progression of patients with PSC with and without concomitant IBD.

#### CONFLICTS OF INTEREST

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