

Review article: how the intestinal microbiota may reflect disease activity and influence therapeutic outcome in inflammatory bowel disease

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Summary

Background: Intestinal bacteria produce metabolites and by-products necessary for homeostasis. Imbalance in this equilibrium is linked to multiple pathologies including inflammatory bowel disease (IBD). The role of the gut microbiota in determining treatment response is becoming apparent, and may act as biomarker for efficacy.

Aim: To describe knowledge about the intestinal microbiota on disease severity and treatment outcomes in IBD

Methods: Descriptive review using PubMed to identify literature on the intestinal microbiota in IBD

Results: Severe IBD has a less diverse microbiota with fewer commensal microbiota communities and more opportunistic pathogenic bacteria originating from the oral cavity or respiratory tract. IBD treatments can alter gut microbiota composition, but in vitro/in vivo studies are needed to prove causation. A diversification of the microbiota is observed during remission. Patients with a more diverse baseline microbiome and higher microbial diversity show better response to anti-tumour necrosis factor- α , vedolizumab and ustekinumab therapy. Higher abundance of short chain fatty acid-producing bacteria, fewer mucus-colonising bacteria and lower abundance of pro-inflammatory bacteria have also been associated with a favourable outcome. Predictive models, based on a combination of microbiota, clinical data and serological markers, have good accuracy for treatment outcome and disease severity.

Conclusion: The intestinal microbiota in IBD carries a set of promising biomarkers of disease activity and prediction of therapeutic outcome. Current insights may also help in designing microbiota modulation strategies to improve outcomes in IBD.

The Handling Editor for this article was Professor Jonathan Rhodes, and this uncommissioned review was accepted for publication after full peer-review.

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1 | INTRODUCTION: THE URGENT NEED FOR BETTER THERAPEUTIC PREDICTION IN IBD

Deviations in the interaction between the innate and adaptive immune system and the intestinal microbiota are increasingly shown to contribute to the onset of inflammatory diseases, such as gingivitis, clostridium difficile infection, obesity, diabetes mellitus type 1, pulmonary disease, colorectal cancer, rheumatoid arthritis, IBD, cardiovascular disease and non-alcoholic fatty liver disease.^{1–10} Several factors may modify or dysregulate the intestinal immune system including dietary regimens, antibiotic treatments and pathogen invasions, which in turn cause a widespread disruption of the microbial composition community. Authors are trying to indicate that the relationship works both ways.

The last two decades have been marked by significant therapeutic advances in the field of IBD, mainly with the approval of targeted biological agents but also in microbiota-modulating therapies such as diet, faecal microbiota transplantation, pre- and probiotics and antibiotics.¹¹ Three distinct therapeutic classes (anti-tumour necrosis factor alpha [anti-TNF α], anti-integrin and anti-interleukin [IL] 12/23 therapy) have received regulatory approval, and recently, tofacitinib, a small molecule belonging to the family of Janus kinase inhibitors, was added to the therapeutic arsenal. Biologic therapy has enabled the expansion of treatment goals to clinical steroid-free remission and endoscopic healing, and more recently, histological remission has shown to be a reachable target in ulcerative colitis (UC). However, these biological therapies have also significantly increased healthcare expenses. Park *et al*¹² demonstrated that—while fewer than 20% of IBD patients were receiving biologic therapies—this subset of the population incurred two to three times the total costs of care per year compared with patients not receiving biologic therapies. There is therefore a pressing need for cost-effective strategies and lifestyle changes could be one of them.

1.1 | Variability in drug response

Inter-individual differences in response to a specific drug can affect either the efficacy and/or toxicity of the treatment. Response rates to mesalazine (mesalamine) or 5-aminosalicylic acid used in UC, are 60%, indicating that 40% of patients may not experience any benefit or may even suffer from adverse drug reactions.¹³ Primary non-response has also been observed in 20%–30% of the patients receiving anti-TNF α , vedolizumab and/or ustekinumab therapy. Incomplete patient assessment preceding the start of a biological therapy may partially explain this high rate of non-response. Accurate prediction of responsiveness prior to therapy start would be of great value, but clinical predictors have proven insufficient and targeted assays are still lacking.

The inter-individual variability in drug response affects not only patient's well-being, it also poses an enormous financial burden.

A variety of factors, among which genetic background, physiological status (eg gender, age, concomitant diseases, starvation and circadian rhythm) and environmental contributors (eg co-administered medications, diet, smoking behaviour and environmental pollutants) may all impact drug response. These multi-factorial contributors have been particularly well studied for anti-TNF α medication in IBD, as summarized in Figure 1. Genetic markers have been extensively scrutinized in attempt to predict response to infliximab or other anti-TNF α medication, but no predictive signature has successfully been validated. The large (N = 1610) PANTS (Personalized anti-TNF α therapy in Crohn's disease) cohort of Crohn's disease (CD) patients naive to anti-TNF α therapy (UK; ClinicalTrials.gov identifier: NCT03088449) demonstrated that the human leukocyte antigen allele, HLA-DQA1*05, carried by approximately 40% of Europeans, is associated with a twofold risk of developing immunogenicity against anti-TNF α therapies, leading to loss of response.^{14–18} Pre-treatment genetic testing for HLA-DQA1*05, may therefore help personalising therapy choice in IBD patients, including taking the necessary precautions to prevent immunogenicity in patients at risk.

Less well studied is the effect of the microbiota—especially the gut microbiota—in predicting response to therapy. Pharmacomicrobiomics is an emerging field that investigates the interplay of microbiome variation and drug response and disposition (absorption, distribution, metabolism and excretion). The gut microbiota can, for example, influence cancer pathogenesis and its therapeutic outcome by metabolising anti-tumoral compounds, or by modulating the host's immune response and inflammation pathways.¹⁹

1.2 | Patient stratification in IBD: the key to a personalized medicine strategy

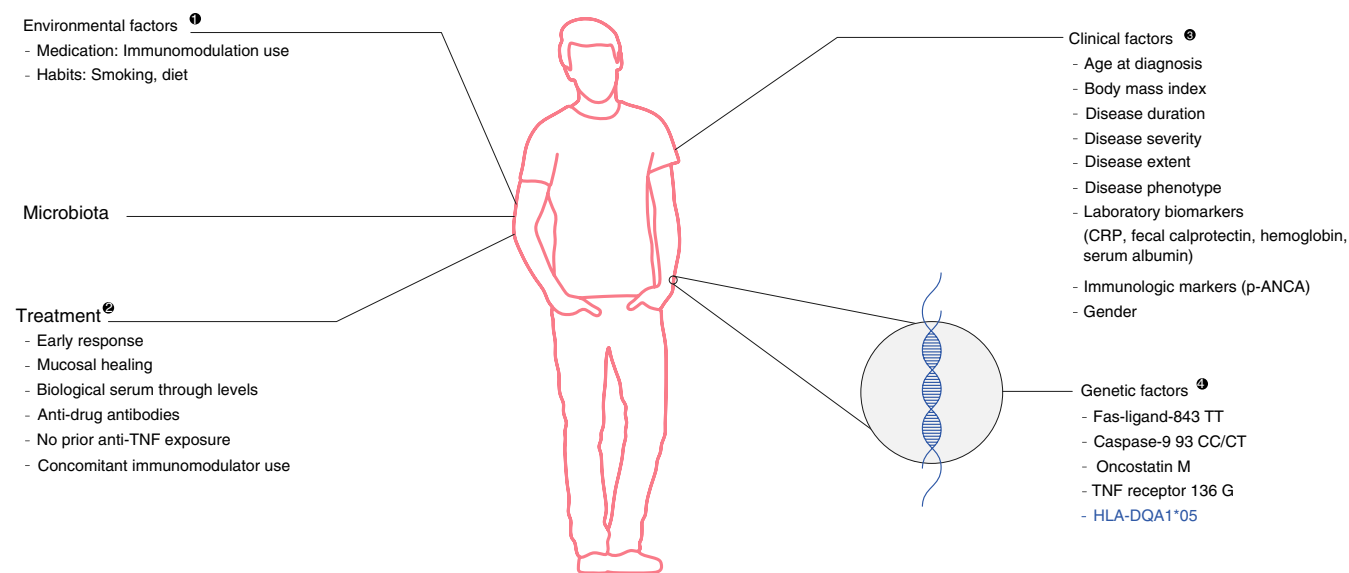
Personalized medicine is tailoring medical treatment to the individual characteristics of a patient.

It relies on the identification of signatures for accurate patient stratification.

In IBD, until recently, this meant identifying genetic, clinical and environmental information, which allowed advances in our understanding of how a patient's unique portfolio makes them vulnerable to certain diseases and disease phenotypes. The personalized medicine approach, as opposed to the classical approach ("one size fits all"), aims to increase our ability to predict which medical treatments will be safe and effective for which patients, and which ones will not, to reduce financial and time expenses, and ultimately increase quality of life. The adoption of personalized medicine in IBD would not only facilitate treatment choice (no treatment, biological treatment, immunomodulators, etc), enhance treatment efficacy, but also allow an earlier detection of possible treatment side effects.^{20,21}

The gut microbiota has more recently been proposed as a potential biomarker and predictor that should be added to a patient's stratification to address some of the above problems.

Factors impacting anti-TNF response



①

Wils et al.⁹⁵ – 2014
 Cohen et al.¹¹⁵ – 2012
 Juillerat et al.¹¹⁶ – 2015
 Rusman et al.¹¹⁸ – 2018
 Olivera et al.¹¹⁹ – 2017

②

Lopetuso et al.¹²⁰ – 2017
 Ferrante et al.¹²¹ – 2008
 Taxonera et al.¹²² – 2011
 McDermott et al.¹²³ – 2013
 Colombel et al.¹²⁴ – 2011
 Gustavsson et al.¹²⁵ – 2010
 Sandborn et al.¹²⁶ – 2014
 Roblin et al.¹²⁷ – 2014
 Vande Casteele et al.^{128 & 129} – 2013 en 2015

③

Lopetuso et al.¹²⁰ – 2017
 Molander et al.¹³⁰ – 2017
 Juillerat et al.¹¹⁶ – 2015
 Reinisch et al.¹⁴⁰ – 2011
 Harper et al.¹³¹ – 2013
 Vermeire et al.¹³² – 2002
 Moran et al.¹³³ – 2014
 Silverberg et al.¹³⁴ – 2005
 Esters et al.¹³⁵ – 2002
 Oussalah et al.¹³⁶ – 2010

④

Lopetuso et al.¹²⁰ – 2017
 Hlavaty et al.^{15 & 137} – 2007 en 2005
 West et al.¹³⁸ – 2017
 Sazonovs et al.¹⁸ – 2020

Legend

TNF : Tumor necrosis factor

CRP: C-reactive protein

p-ANCA: perinuclear-anti neutrophil cytoplasmic antibodies

HLA: Human leukocyte antigen

FIGURE 1 Genetic, environmental treatment (concomitant medication and treatment-related factors (eg trough levels)) and clinical factors impacting anti-TNF α therapy response in IBD.^{15,112–137}

To be truly valuable, biomarkers need to be reproducibly more accurate, safer and/or cheaper than the biomarkers or procedures that we currently use to make these decisions (eg serum C-reactive protein [CRP], faecal calprotectin [Fcal], endoscopy, histology, genetic screening, etc). Such a high standard patient stratification tool will likely require the assessment and integration of different layers of -omics information from the patients (metagenomics, metabolomics, genomics, proteomics, etc.), supplemented with high-resolution phenotype data, to achieve a successful personalized medicine strategy.

Multiple research groups and consortia have in recent years joined forces and are generating datasets combining clinical data with genomic data, proteomic data, transcriptome data or gut microbiome data. These datasets hold the hope to unravel promising biomarker signatures for IBD treatment in the future.

2 | IBD AND GUT MICROBIOTA

The importance of the microbiota in IBD was first suggested by genome-wide association studies, which identified human genes in pathways related to recognition of bacterial peptides and elimination of intracellular bacteria, which were linked with a higher risk of IBD development.²² Moreover, Rutgeerts *et al* demonstrated in humans that faecal stream diversion prevents post-operative recurrence of CD²³ and the intestinal microbiota is essential for the development of inflammation in animal models of colitis. Germ-free rodent colitis models, used as models for IBD, have no intestinal inflammation, but rapidly develop colitis and pathogenic immune responses after colonisation with specific non-pathogenic enteric bacteria.²⁴ Antibiotics are able to

induce remission in active CD patients and are in the prevention of post-operative recurrence CD. These studies suggest that the microbiota plays an important role in inducing intestinal inflammation.²⁵ In IBD, it has consistently been shown that there is a general decrease in gut microbiota biodiversity, knowingly α diversity, and in species richness, a measure of the total number of species in a community, particularly in Firmicutes and Bacteroides, and a relative increase of other bacterial species, for example, the ones belonging to the Enterobacteriaceae.^{26,27} However, at present, no single pathogenic bacterium has been identified as a causative agent of IBD. IBD patients do have fewer bacteria with protective properties (*Bifidobacterium* species, *Bacteroides* and *Clostridium* Groups IV and XIVA with *Faecalibacterium prausnitzii* and *Roseburia* species) and more with pro-inflammatory properties (Veillonellaceae, Pasteurellaceae, *Escherichia coli* (adherent/invasive), and *Fusobacteriaceae*).^{28–31} Such a shift of the gut ecosystem, which contributes to, promotes or sustains pathogenic states, is referred to as dysbiosis, as opposed to eubiosis, the normal microbiota composition.

Both CD and UC gut microbiomes exhibit important changes relative to healthy gut microbiomes. In CD, proportions of the Clostridia family are altered: the *Roseburia* and *Faecalibacterium* genera of the Lachnospiraceae and Ruminococcaceae families are decreased, whereas *Ruminococcus gnavus*, *Escherichia coli*, increased.

Alterations in faecal concentrations of microbial metabolites have also been described in IBD patients with reduction of medium- and short-chain fatty acids (SCFAs), dysregulation of bile acid derivatives and tryptophan metabolites.³² SCFAs affect the differentiation and expansion of Treg cells and the growth of epithelial cells,³³ known to play a central role in maintaining intestinal homeostasis. An increase in sulphate-reducing bacteria, as *Desulfovibrio piger*, has also been described resulting in higher hydrogen-sulphate that may harm intestinal epithelial cells and induce mucosal inflammation.^{34–37} Metabolomics certainly will become an important adjunct in the clinical assessment and management of IBD, but are beyond the scope of this review.

2.1 | Monitoring IBD disease activity: the gut microbiota as IBD disease activity biomarker

In IBD, monitoring mucosal inflammation is crucial to limit disease progression and complications.

Endoscopy is the current gold standard for this but is invasive, and clinical activity scores poorly correlate with this gold standard. Faecal and serologic biomarkers of gastro-intestinal inflammation have been introduced as relatively inexpensive alternatives to diagnose and follow-up disease activity. FCal, a neutrophil cytosolic protein, and serum CRP are considered to be the best non-invasive surrogate markers to date.

Dysbiosis has been associated significantly with clinical and biological measures of disease severity. Clear differences have

been reported in active vs quiescent disease, although results between studies are inconsistent, most likely due to methodological differences (real-time polymerase chain reaction, denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, fluorescence in situ hybridisation, temporal temperature gradient gel electrophoresis or next-generation sequencing including 16S ribosomal RNA gene sequencing and shotgun sequencing).

The factors responsible for triggering flares are largely unknown. Higher levels of gut inflammation in IBD patients have been associated with reduced microbial richness (or lower α -diversity), reduced abundance of SCFA producers, increase in bacteria typical of the oral cavity (*Veillonella dispar*, *Aggregatibacter segnis*, *Campylobacter*, Lachnospiraceae, *Veillonella parvula*, *Haemophilus parainfluenzae*, *Megasphaera*), and reduced relative abundance of Gram-positive bacteria.^{30,37–40} Increased oxygen levels in the gut may further favour expansion of aerotolerant bacteria from the oral cavity and facilitate strain-specific niche adaptation.

Several studies have proposed microbiota signatures of disease activity, but so far, no specific bacterial taxa have been identified. Investigating the microbial community structure (a combination of operational taxonomic units [OTUs]) rather than a specific microbial taxa might be more effective in investigating the role of the intestinal microbiota in IBD. These attempts can be grouped in two main strategies: (a) a summary statistic or stratification of the microbiota (eg microbiota diversity, species richness or enterotypes), or (b) a signature comprised of specific taxa (single or multiple) based on an increase/decrease in relative abundance of specific species in active IBD compared with IBD patients in remission (Figure 2).

None so far have proven any causation between disease severity and the gut microbiota. Indirect experimental models are needed to establish causal relationships between altered microbiomes and pathologic conditions. The most commonly used model to make such causal inferences is the transplantation of faecal microbial communities from individuals with and without a disease/disease state into germ-free rodents, followed by a comparative analysis of pathological phenotypes in the recipient animals.

The majority of the included studies report an enumeration of taxa that are negatively or positively associated with disease activity in CD/UC (most of the time not specified), measured in most of the studies by clinical activity scores or faecal and/or serological biomarkers (CRP or FCal)—Table 1 and Figure 2.^{30,38,39,41–51}

2.2 | Fusobacterium

Fusobacterium has been described to be increased in active IBD (Figure 2).^{39,52,53}

Fusobacterium is a genus of obligatory anaerobic filamentous Gram-negative rods, and members of the phylum Fusobacter. *Fusobacterium* is a common member of the oral microbiota and can have a symbiotic relationship with its host, but is famous to cause opportunistic infections. It is rapidly gaining notoriety as a pathogen

(A) Increased in inactive IBD/Decreased in active IBD (relative abundances) (B) Increased in active IBD/Decreased in inactive IBD (relative abundances)

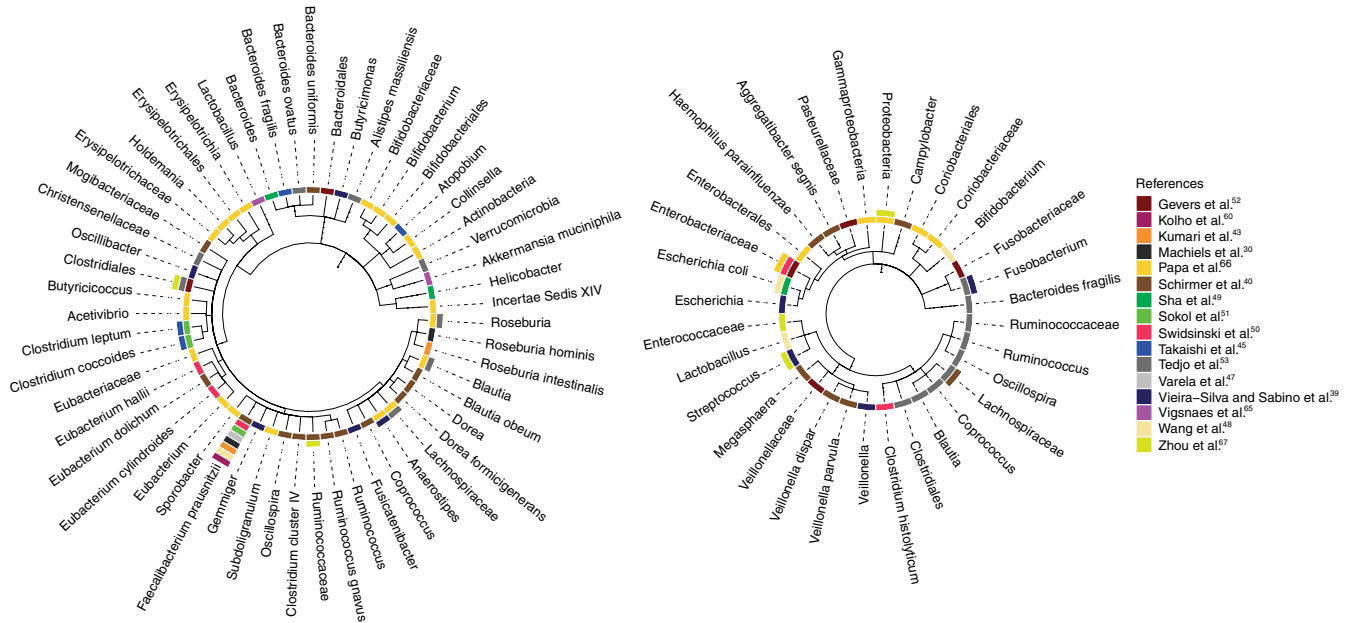


FIGURE 2 Cladogram summarising previously established links between the gut microbiome and IBD disease activity.^{30,39,40,43,45,47–53,60,65–67} Cladogram listing all bacterial taxa reported to be associated with IBD disease activity in human studies. Bacterial taxa are labelled (coloured ring) according to publication origin (coloured dots). The colours used for the included studies, correspond with the coloured dots used in Table 1

with a surprising number of associated diseases. It has been associated with post-operative recurrence in CD patients after ileocecal resection,⁵⁴ with a worse outcome after faecal microbiota transplantation in UC patients⁵⁵ and recently, in CD patients with primary sclerosing cholangitis.^{39,56}

2.3 | Enterobacteriaceae

Enterobacteriaceae is a large family of Gram-negative bacteria. It includes, along with many harmless symbionts, many of the more familiar gastrointestinal pathogens, such as *Salmonella*, *Escherichia coli*, *Klebsiella* and *Shigella*.

Escherichia coli colonizes the intestinal tract of human infants immediately after birth and helps to maintain normal intestinal homeostasis. However, in the pathogenesis of IBD, the concept of the defective innate immune function, may result in a diminished bacterial killing and functional alterations of the commensal bacteria, like *E. coli*. Darfeuille-Michaud et al.^{57,58} described that the Adherent-Invasive *E. coli* is specifically associated with the ileal mucosa in CD in 2004, while the diffusely adherent *E. coli* has been associated with UC. Kotłowski et al.⁵⁹ demonstrated that the mucosa associated microbiota of IBD patients contained higher counts of *E. coli* of the virulent B2 groups than controls.

This may suggest that IBD-associated *E. coli* strains could play an important role during IBD flares.

As is the case with adherent-invasive *E. coli* and CD, the link between diffusely adherent *E. coli* or B2 *E. coli* and UC is associative, and no data so far reveal a causative link.

2.4 | Clostridium leptum (Clostridium cluster IV) and Clostridium coccoides/Eubacterium rectale (Clostridium cluster XIVa)

Clostridium leptum and *coccoides* (Firmicutes phylum), are major players in the human microbiome (Figure 2).^{30,39,43,45,47,48,50,51,60} They represent 10%–40% of the total bacteria.^{61,62} They are indispensable regulators of intestinal homeostasis. The *C. leptum* group, including *F. prausnitzii*, and certain species of *Eubacterium* and *Ruminococcus*, and the *Clostridium coccoides* group, including *Roseburia intestinalis*, are both butyrate-producing bacteria. Butyrate (short chain fatty acid) is a major source of energy for colonic epithelial cells and exhibits immunomodulatory and anti-inflammatory potential. Hence, the decrease in butyrate producing bacteria from the *C. leptum* group and *C. coccoides* could play a role in the onset of IBD flare-ups.

Faecalibacterium prausnitzii, the major bacterium of the *C. leptum* group, is one of the most abundant anaerobic bacteria in the human intestine. The concentration of *F. prausnitzii* in the faeces of IBD patients is significantly lower than in healthy controls.³⁰ Lower proportions of *F. prausnitzii* in the resected ileal Crohn mucosa, are associated with a higher risk of postoperative recurrence.⁶³ It is thought that *F. prausnitzii* attenuates intestinal inflammation by secreting certain yet unidentified anti-inflammatory substances.⁶³ One particular strain of this species—A2-165—demonstrated a strong capacity to induce IL-10 in human and murine dendritic cells and influence T cell differentiation in vitro and in vivo. The reduction in interferon- γ^+ cells in vivo and the IL-10 induction in the lamina propria cells of damaged intestinal tissue could be a mechanism contributing to the suppressive effect on inflammation in mouse colitis models.⁶⁴

TABLE 1 Characteristics of included studies concerning the gut microbiota in disease activity^{30,38–41,43–53,60,65–67}

	CD	UC	Population	Design	Specimen	Microbiology assessment	Measure of disease activity
● Swidsinski <i>et al</i> ⁵⁰	82	105	Adult	Prospective	Feces	FISH	CDAI and SCCAI
● Takaishi <i>et al</i> ⁴⁵	23	73	Adult	Cross-sectional	Feces	qPCR and FISH	Clinical symptoms (blood loss) and endoscopic inflammation
● Sokol <i>et al</i> ⁵¹	32	17	Adult	Cross-sectional	Feces	qPCR	CDAI and SCCAI
● Papa <i>et al</i> ⁶⁶	23	44 (1 IBDU)	Pediatric and adult	Cross-sectional	Feces	16S rRNA sequencing (V1–V3)	PUCAI and PCDAI
● Vignaers <i>et al</i> ⁶⁵		12	Adult	Cross-sectional	Feces	qPCR and DGGE	Based on clinical and endoscopic assessment
● Kumari <i>et al</i> ⁴³		26	Pediatric and adult	Cross-sectional	Feces	qPCR and FISH	SCCAI
● Sha <i>et al</i> ⁴⁹		26	Adult	Cross-sectional	Feces	qPCR and DGGE	SSCAI
● Varela <i>et al</i> ⁴⁷		19	Adult	Prospective	Feces	RTqPCR	SCCAI
● Gevers <i>et al</i> ⁵²	447		Pediatric	Cross-sectional	Mucosal biopsies	16S rRNA sequencing (V4) and shotgun sequencing	PCDAI
● Machiels <i>et al</i> ³⁰		127	Adult	Cross-sectional	Feces	DGGE analysis	Partial Mayo subscore
● Rajca <i>et al</i> ⁴⁴	33		Adult	Prospective	Feces	qPCR	CDAI
● Wang <i>et al</i> ⁴⁸	21	34	Adult	Cross-sectional	Feces and mucosal biopsies	qPCR	CDAI and UCAI
● Kolho <i>et al</i> ⁶⁰	36	26	Pediatric	Prospective	Feces	Phylogenetic microarray (HITchip) and qPCR	Fecal calprotectin
● Tedjo <i>et al</i> ⁵³	71		Adult	Prospective	Feces	16S rRNA sequencing (V1–V3)	Fecal calprotectin, HBI and CRP
● Shaw <i>et al</i> ⁴¹	15	4	Pediatric	Prospective	Feces	16S rRNA sequencing (V4)	Fecal calprotectin
● Doherty <i>et al</i> ⁴⁶	232		Adult	Prospective	Feces	16S rRNA sequencing (V4)	CDAI
● Schirmer <i>et al</i> ⁴⁰		405	Pediatric	Prospective	Feces and mucosal biopsies	16S rRNA sequencing (V4)	PUCAI
● Vieira-Silva and Sabino <i>et al</i> ³⁹	49	39	Adult	Cross-sectional	Feces	16S rRNA sequencing (V4)	Fecal calprotectin
● Franzosa <i>et al</i> ³⁸	68	53	Adult	Cross-sectional	Feces	Shotgun sequencing	Fecal calprotectin
● Zhou <i>et al</i> ⁶⁷	72	51	Adult	Prospective	Feces	16S rRNA sequencing (V4)	Mayo and CDAI

The coloured dots before the included studies in the table correspond with the colours used in Figure 2.

Abbreviations: CD, Crohn's disease; HBI, Harvey bradshaw index; HITchip, Human intestinal tract chip; IBD, Inflammatory bowel disease; NR, Non-responder; PCDAI, Pediatric Crohn's disease activity index; PUCAI, Pediatric Ulcerative colitis activity index; qPCR, quantitative polymerase chain reaction; R, responder; rRNA, ribosomal ribonucleic acid; SCCAI, simple clinical colitis activity index; UC, ulcerative colitis.

2.5 | Bifidobacterium and Lactobacillus

Both increased and reduced (in most data) relative abundance of *Bifidobacterium* and *Lactobacillus* have been described in active IBD (Figure 2).^{40,48–51,65–67}

Bifidobacterium and *Lactobacillus* are an important part of our normal intestinal microbiota and are known as the best characterized and widely commercialized probiotics. They are both non-spore-forming, Gram-positive, lactic acid-producing bacteria. *Lactobacilli* have limited biosynthetic abilities and ferment sugars, generating

lactic acid as major end product, whereas *Bifidobacteria* are important producers of short-chain fatty acids.

The effect of probiotic administration is difficult to interpret between studies as the dose used, the frequency and duration of use, and the use of concomitant treatment differs. More controlled trials using harmonisation in methodology are needed.

2.6 | Veillonella

Veillonella are Gram-negative anaerobic cocci and belong to the family Veillonellaceae. Strains detected in oral cavities include *V parvula*, *Veillonella atypic*, and *V dispar*.

Veillonella dispar has been described as the OTU with the largest decrease between mild and severe disease by Schirmer *et al* (Figure 2).

Enrichment of *Veillonella* species has been described in CD, in PSC and other fibrotic disorders.^{68–70}

2.7 | Dysbiosis indexes, enterotyping and microbial modelling

Gevers *et al* made the first attempt to translate microbiota results into a microbial dysbiosis index (MD-index), in paediatric CD. The dysbiosis index was defined as the ratio between the total relative abundance in organisms increased in mucosal biopsies (*Veillonella*, *Haemophilus*, *Escherichia* and *Fusobacterium*) over the total relative abundance of organisms decreased in mucosal biopsies of paediatric CD (*Dialister*, *Bilophilus*, *Oscillospira*, *Faecalibacterium*, Ruminococcaceae, *Dorea*, Erysipelotrichaceae, *Ruminococcus*, *Coprococcus*, Lachnospiraceae, *Bacteroides*, *Parabacteroides*, Rikenellaceae and *Sutterella*). The authors showed that this MD-index correlated well with levels of inflammation based on Paediatric CD Activity Index (PCDAI) and faecal calprotectin and had a negative correlation with species richness. Shaw *et al* confirmed this correlation with disease severity but failed to find a correlation between the α -diversity (Shannon) and faecal calprotectin and also between the MD-index and treatment response.^{41,52}

More recently, the *Bacteroides* 2 enterotype has been associated with inflammatory biomarkers (CRP and FCal) in IBD and PSC patients.³⁹ *Bacteroides* 2 enterotype has an 80% prevalence in patients suffering from inflammatory pathology like CD and UC.⁷¹

More recent studies used multivariate analysis or machine learning (random forest-based) algorithms to identify more complex signatures based on multiple species rather than individual species as markers for disease activity.

Kolho *et al*, Tedjo *et al* and Papa *et al*, all developed models combining bacterial groups relative abundances, respectively a combination of 9, 50, 28 bacterial taxa, that predict inflammation as measured by FCal, PCDAI, PUCAI, Harvey Bradshaw index, with an area under the curve (AUC) value of 0.85, 0.82, 0.72 respectively.^{53,60,66} The taxa in these models include both commensal microorganisms as well as opportunistic pathogens, further indicating that merely detecting presence

or absence of specific taxa is not sufficient. The most discriminative taxa overlap to a significant degree and include several bacterial clades that had previously been associated with disease activity in IBD, including the family Lachnospiraceae with the genus *Roseburia*, the Proteobacteria phylum, specifically the Gammaproteobacteria class where under *Serratia*, *Escherichia-Shigella* and the Corynebacteriaceae family in severe disease and the genera *Ruminococcus*, *Blautia*, *Faecalibacterium* and species *Bacteroides fragilis*.

In summary, the microbiota of active IBD patients is less diverse and rich^{46,60} than the microbiota of IBD patients in remission. This indicates a diversification of microbiota composition in IBD patients during remission, as is observed in healthy controls,⁷² while during exacerbation patients converge towards a more similar dysbiotic profile, where commensal microorganisms can become opportunistic.³⁹ A striking decrease of core gut microbes from the Firmicutes phylum, such as the SCFA-producers *C leptum* and *coccoides* and increase in bacteria typical of the oral cavity in patients with more severe disease was observed. Healthy guts may be naturally resistant to colonisation by bacteria from the oral cavity, but inflammation or strain-specific adaptation, including antibiotic resistance and virulence genes such as adhesion genes, may allow these microbes to colonize the gut mucosa in IBD and exacerbate inflammation. Several bacterial taxa are overlapping between the different studies including *Fusobacterium*, *Escherichia coli*, *C leptum* and *coccoides*, *Bifidobacterium*, *Lactobacillus* and *Veillonella* but the combination of different OTUs will become more essential in the future, rather than the search for one specific (causative) bacterial taxa. The identified correlations between the faecal microbiota and IBD activity may allow to even better monitor disease activity in the future.

Future studies should compare new microbiome-derived indexes or (multivariate) models with intestinal inflammation, based on endoscopic (or even better histologic) examination.

Almost all studies so far used FCal instead of correlating with gold standard endoscopic data. In order to be implemented in routine daily practice, indexes should not have more than 50 taxa and should enable fast and less expensive results.

3 | IBD TREATMENT AND GUT MICROBIOTA

Falony *et al*⁷² showed that in an average section of the Flemish population (Flemish Gut Flora Project), nearly 10% of inter-individual variation in gut microbiome can be explained by medication use. In IBD, it remains unknown what portion of the alterations observed in gut microbiota are attributable to medication, intestinal inflammation or other disease symptoms. Our understanding of drug metabolism and response in IBD is limited. Gaining insights into IBD-specific modifications of drug metabolism by the gut microbiota, may allow for improved bioavailability and efficacy of the administered drug. Orally ingested drugs pass through the upper gastro-intestinal tract and small intestine into the large intestine, where they come across thousands of microbial species that reside in the human gut. Complex drug-microbial interactions can occur in the colon. Drugs may change intestinal

microenvironment, alter microbial community composition (eg Proton pump inhibitors)⁷³ or affect bacterial growth (eg Metformin).⁷⁴ The gut microbiome may also participate directly in chemical transformation of drugs (eg sulfasalazine⁷⁵ and digoxin⁷⁶). Bacterial cleavage of azo bonds in sulfasalazine can favourably achieve site-specific release of the anti-inflammatory sulfapyridine and 5-aminosalicylic acid.

To what extent IBD therapies are able to interfere with gut microbial community structures is not known. It was shown in cancer therapy that the efficacy of targeted therapies, such as immunotherapy as well as classical chemotherapy, can be modulated by gut microbial community composition,^{77,78} in IBD this has not been shown (yet).

We next describe the effect of IBD medication on our gut microbiota and the potential of gut microbiota profiles to act as predictors of therapy success.

4 | THE GUT MICROBIOTA AS PREDICTORS OF TREATMENT SUCCESS IN IBD

4.1 | Biologicals

Biologic therapies such as monoclonal antibodies are classically acquired from living cell lines using recombinant DNA technology. They target mediators in crucial immunological and inflammatory pathways, allowing selective but highly potent regulation.

Anti-TNF α agents were the first class of biologic agents approved for use in IBD. Although anti-TNF α agents are highly effective in treating luminal and extra-intestinal manifestations of disease, around 25% of patients are primary non-responders and another 30% of patients lose response over time, the so-called secondary non-responders. While secondary loss of response is often associated with immunogenicity and the formation of neutralising antibodies, the reasons underlying primary non-response are poorly understood. In both IBD as in spondyloarthritis, a lower

baseline microbiota diversity (α -diversity) is associated with anti-TNF α non-responders and lower dysbiosis indexes with responders although results are not unanimous.^{41,60,79–81} The complexity in predicting treatment response using the intestinal microbiota is highlighted by the poor separation between patients in remission and non-remitters in a simple principal component analysis in all of the published studies. Integration of clinical and endoscopic or biochemical factors with data on the microbiome or integration of multi-omics will probably be necessary to increase accuracy. Some studies have already combined clinical and microbial data, and suggested a complementary role for both.^{37,82}

Few longitudinal studies related the intestinal microbiota to anti-TNF α treatment response. A first study by Kolho *et al* in 11 paediatric IBD patients (six responders, five non-responders, not specified whether UC or CD patients) examined anti-TNF α treatment response at week 6 and used FCal as outcome measure. A higher relative abundance at baseline of five groups of bacteria, including *Bifidobacterium*, *Clostridium colinum* (*Clostridium cluster IV*), *Eubacterium rectale*, uncultured Clostridiales, and *Vibrio*, and a lower relative abundance at baseline of *Streptococcus mitis*, predicted response to anti-TNF α medication (Figure 3).⁶⁰ In contrast, two other prospective studies, one in 17 paediatric CD and UC patients⁶⁰ and another one in 16 CD patients,⁶⁷ defined long-term response using endoscopic criteria. Both studies used random forest classifier based models and predicted anti-TNF- α treatment response with respective areas under the curve of 0.75 and 0.87. Zhou *et al* was able to increase the accuracy to 0.94 by combining microbiota data with faecal calprotectin and CDAI data, with multiple *Clostridiales* OTUs as most informative feature contributing to the prognosis model.⁶⁷

Two of the predictive signature bacteria for response by Kolho *et al* more specifically *Bifidobacterium* and *E. rectale* have been associated with health and are known to have immune dampening effects. Similarly, also *Faecalibacterium* is known to exert these properties. Interestingly, two independent studies from the Emory and Gothenburg University demonstrated a depletion of *F. prausnitzii*

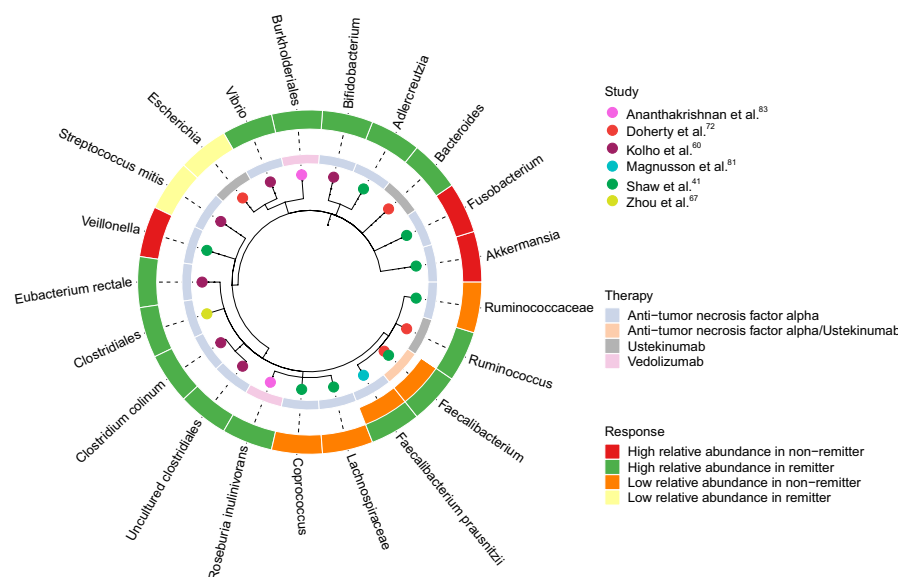


FIGURE 3 Cladogram summarising previously established links between the gut microbiome and biologic treatment outcomes in IBD patients.^{41,46,60,67,80,82}

Cladogram listing all bacterial taxa reported to be associated with response to biologic therapy in human studies. Bacterial taxa are labelled according to publication origin (coloured dots). The inner ring represents the biologic therapy used in the published study (Therapy), while the outer ring (Response) whether the taxa were associated with response or non-response. The colours used for the included studies, correspond with the coloured dots used in Table 2

TABLE 2 Characteristics of included studies concerning the gut microbiota and biologic treatment outcomes in IBD^{41,46,60,67,80–82}

Study	IBD (R/ NR)	CD (R/ NR)	UC (R/ NR)	Population	Design	Specimen	Microbiology assessment	Outcome measure
● Kolho <i>et al</i> ⁶⁰	6/5			Pediatric	Prospective	Feces	Phylogenetic microarray (HITchip) and qPCR	Fecal calprotectin
● Magnusson <i>et al</i> ⁸⁰			4/3	Adult	Prospective	Feces	16S rRNA sequencing (V3-V9)	Mayo score
● Ananthakrishnan <i>et al</i> ⁸²		9/30	22/22	Adult	Prospective	Feces	Shotgun sequencing	HBI and SSCAI
● Shaw <i>et al</i> ⁴¹			5/12	Pediatric	Prospective	Feces	16S rRNA sequencing (V4)	PCDAI/ PUCAI
Aden <i>et al</i> ⁸¹	9/3			Adult	Prospective	Feces	16S rRNA sequencing (V3-V4)	HBI and partial Mayo score
● Doherty <i>et al</i> ⁴⁶		31/ 201		Adult	Prospective	Feces	16S rRNA sequencing (V4)	CDAI
● Zhou <i>et al</i> ⁶⁷		16 in total		Adult	Prospective	Feces	16S rRNA sequencing (V4)	CDAI

The coloured dots before the included studies correspond in the table with the colours used in Figure 3.

Abbreviations: CD, Crohn's disease; HBI, Harvey bradshaw index; HITchip, Human intestinal tract chip; IBD, Inflammatory bowel disease; NR, non-responder; PCDAI, pediatric Crohn's disease activity index; PUCAI, pediatric Ulcerative colitis activity index; qPCR, quantitative polymerase chain reaction; R, responder; rRNA, ribosomal ribonucleic acid; SCCAI, simple clinical colitis activity index; UC, ulcerative colitis.

at baseline in anti-TNF α non-responders compared with responders.^{41,80} However, a subsequent study by Aden *et al*⁸¹ could not confirm these associations possibly due to small sample size.

Faecalibacterium prausnitzii belongs to the same *Clostridium* cluster IXa as *E. rectale*. Low concentrations of *F. prausnitzii* have been correlated with early recurrence of CD after anti-TNF- α treatment interruption.⁴⁴

Bifidobacterium, *E. rectale* and *F. prausnitzii* are all SCFAs producers,⁸³ of which butyrate in particular, regulates innate and adaptive immune cell generation, their function and trafficking.⁸⁴ This important role of SCFA producers (acetate, propionate and more particular butyrate), is a recurring theme in the specialized literature on predicting treatment response.

Not only a reduction of beneficial bacteria but also a higher abundance of (potential) harmful bacteria has been related to response to anti-TNF α . *Fusobacterium* and *Veillonella*, have also been reported to be increased in anti-TNF α non-responders at baseline.⁴¹ Another potential harmful species related to anti-TNF α response is *Akkermansia*. Shaw *et al* demonstrated a higher pre-treatment relative abundance of *Akkermansia* non-responders compared to responders. *Akkermansia* is considered to be a mucus-colonising bacterium. Although *Akkermansia* generally is known for its beneficial properties, overabundance of this species is not always associated with benefits.^{85,86} The mucous layer is a protective lubricant layer of highly glycosylated mucins and serves as the initiation surface for many host-microbe interactions. Various bacteria have developed mechanisms that allow them to adhere to and to use mucus as a source of carbon and energy. In this way, these bacteria do not compete with the microbiota in the highly populated

lumen and do not depend on nutrients deriving from host food consumption.⁸⁷

Finally, not only bacterial species but also the expression patterns of antimicrobial peptides have been related to anti-TNF α response. Magnusson *et al* demonstrated that UC responders and non-responders to anti-TNF α therapy exhibited distinct mucosal antimicrobial peptides expression patterns. Only responders showed detectable expression of DEF5, BPI, ECP, HIST1, RPS19, HMGB1 and HMGN2, proteins with antimicrobial functions and only non-responders showed detectable expression of HDAC1, which inhibits antimicrobial peptides expression.

Vedolizumab, a humanized anti- $\alpha_4\beta_7$ integrin monoclonal antibody, interferes with leukocyte migration to sites of inflammation. The higher baseline microbiota α -diversity and the relative higher abundances of SCFA-producers, as shown in anti-TNF responders, have also been shown in responders to vedolizumab therapy. A study on 85 prospectively recruited IBD patients,⁸² about to initiate vedolizumab therapy, investigated the relationship between treatment response and the gut microbiota composition and function, based on expression patterns of microbial genes (Table 2, Figure 3) Microbial diversity at baseline and two taxa, *Roseburia inulinivorans* (butyrate and propionate producer with anti-inflammatory effects) and the order Burkholderiales, were significantly higher in CD patients who achieved clinical remission at week 14. Several changes in microbial functions (eg branched chain amino acid synthesis, dTDP-L-rhamnose biosynthesis I, L-isoleucine biosynthesis I in CD and colonic acid building blocks biosynthesis and pyruvate fermentation to acetate and lactate II in UC) were associated with response to vedolizumab,

suggesting also a functional component in addition to taxonomic differences. This study thus suggests multiple differences in taxonomic composition, diversity and function as predictors of response to vedolizumab. A predictive model, vedoNet, based on clinical features and relative microbiota abundances and pathways, had an AUC of 0.872 with an >80% true positive discovery rate with < 25% false negative discovery rate.⁸² Remarkably, vedoNet also correctly identified 11 of the 13 patients achieving remission in a validation cohort of 20 patients starting anti-TNF α therapy, hereby demonstrating that the model predicts inflammation and is not treatment specific.

Another model where a SCFA producer played a significant role in predicting response to ustekinumab, was published recently by Doherty *et al* (Table 2, Figure 3) Ustekinumab is a human Immunoglobulin G antibody to the p40 protein subunit of interleukins IL-12 and IL-23. Results from the phase 2 CERTIFI study with ustekinumab, demonstrated that subjects with CD in remission at week 6 could be distinguished from those with active disease by a predictive model based on the relative abundance of baseline microbial taxa (relative abundance of each OTU and α -diversity), and clinical biomarkers (age, sex, current medications, body mass index, disease duration, disease location, faecal calprotectin, faecal lactoferrin, CRP, bowel stricture, and CDAI subscores) with an AUC of 0.844 (specificity, 0.831; sensitivity, 0.774). The most predictive OTUs classified were genera as *Faecalibacterium* and *Escherichia/Shigella*. Also in this trial, the baseline microbiota diversity was significantly ($P = 0.020$) higher in subjects that achieved remission 6 weeks after treatment initiation.⁴⁶

4.2 | Other IBD treatments

There is no literature available on the predictive role of the gut microbiota in therapeutic success of immunosuppressive therapies such as azathioprine, mercaptopurine and steroids or 5-aminosalicylic acids (mesalazine) in IBD.

A small side note can be made about the enzyme thiopurine methyltransferase, necessary to inactivate azathioprine. A small percentage of patients (0.3% has low or undetectable thiopurine methyltransferase enzyme activity and about $\pm 10\%$ are heterozygous for thiopurine methyltransferase gene mutations resulting in intermediate thiopurine methyltransferase activity⁸⁸) have mutations in the enzyme thiopurine methyltransferase that lead to reduced or complete loss of enzymatic activity—these patients may suffer lethal side effects if given azathioprine. Interestingly, thiopurine methyltransferase is evolutionarily conserved and bacterial thiopurine methyltransferase has activity against azathioprine. This is an example of a bacterial enzyme that may inactivate a drug therapy before it reaches host tissue. Although physicians can screen patients for thiopurine methyltransferase inactivating mutations in the human genome prior to prescribing azathioprine, there is currently no test for the abundance or activity of thiopurine methyltransferase in the microbiome.⁸⁹

5 | THE EFFECT OF IBD THERAPY ON THE GUT MICROBIOTA

Many human microbiome studies have focused on correlating disease states to gut microbial community structure. These cross-sectional studies are challenging to interpret due to the many confounding factors, including the treatment itself.

5.1 | Biologicals

Modifications of microbiota composition by anti-TNF α inhibitors could be caused either by indirect or direct effects. These treatments are well recognized to heal and profoundly down-regulate inflammation in the injured gastro-intestinal mucosa, therefore restoring normal structure of digestive epithelium and tolerance functions towards mucosal microbiota.⁹⁰ In this way, they could indirectly change microbiota composition.

Direct action on the gut microbiota, *via* an inter-kingdom interaction, has been described.⁹¹ The existence of membrane-bound bacterial receptor to TNF α has been suspected long ago, especially on Gram-negative bacteria.⁹²

Although multiple studies describe a restoration of the decreased baseline microbial diversity, shifting towards a healthy control microbiota profile, during biological treatment, sometimes also in non-remitters,⁷⁹ others describe no consistent changes in bacterial groups during anti-TNF α treatment.^{60,79,80}

Doherty *et al* tested whether ustekinumab treatment altered the microbiota based on the subject's response 22 weeks after therapy. The study included 48 subjects who were induced and maintained with ustekinumab therapy (18 responders and 30 non-responders) and 14 subjects induced and maintained with placebo (eight responders and six non-responders). There was no significant difference in the α -diversity over time in subjects who did not respond 22 weeks after induction, regardless of the treatment arm.⁴⁶

Large groups of bacteria do change according to therapeutic outcome, as described above, declaring that there may not be a significant effect of the biological therapy itself on the gut microbiota in IBD.

5.2 | Immunosuppressives

A prospective follow-up cohort of IBD outpatients demonstrated that thiopurines affect the gut microbial composition and diversity in IBD.⁹³ Azathioprine and mercaptopurine treatment increases the concentration and adherence of mucosal bacteria and inhibit the growth of several IBD-linked bacteria such as *Campylobacter concisus* and *Mycobacterium avium* subspecies *paratuberculosis* *in vitro*.^{94,95} Failure of azathioprine and mercaptopurine is therefore hypothesized by some to be caused by insufficient inhibition of these IBD-associated bacterial species.⁹⁶

The mode of thiopurine delivery (oral administration) has also been suggested as a reason of failure for thiopurines, since the conversion of thioguanine into the therapeutically active 6-thioguanine nucleotides, can occur independently of lymphocytes via colonic bacteria. Promoting a more local delivery of thioguanine to sites of intestinal inflammation in IBD could increase therapy success.⁹⁷ Oral methotrexate does not significantly alter the intestinal microbiota in rheumatoid arthritis patients,⁹⁸ but no studies in IBD have been conducted so far.

5.3 | Microbiota and mesalazine

Mesalazine can influence intestinal composition, and decrease bacterial richness and diversity.⁹⁹ Four different hypotheses have been suggested on how mesalazine influences the intestinal flora: (a) A change in colonic luminal pH whereby the growth of *Bifidobacteria* and *Lactobacilli* is enhanced. (b) Improvement of the anoxic environment by inhibiting the production of chemotactic eicosanoids and cyclo-oxygenase 2 (COX2) and inactivation of oxygen-derived free radicals, hereby affecting the composition of the intestinal microbiota. (c) Inhibition of destruction/translocation of tight junction proteins hereby enhancing the mucosal barrier. (d) Direct inhibition of the growth of sulphate-reducing bacteria, for example, *Salmonella enterica* and *C. concisus*.^{96,100} It has been shown that IBD patients who do not use mesalazine have higher faecal sulphide levels.¹⁰¹

5.4 | Microbiota and steroids

The effect of glucocorticoids on the gut microbiota has been investigated in animal models. Rodents treated with a single high dose of dexamethasone show an increase in ileal anaerobic bacteria, while a low dose of glucocorticosteroids increases the coliform bacteria.¹⁰² Chronic exposure to dexamethasone shifted the gut microbiota in rodents in varying degrees to regulate colonic mucin gene expression under both healthy and diseased conditions.¹⁰³ Doherty *et al* demonstrated that corticosteroid use in IBD patients was associated with 1.45-fold higher α -diversity and also the β -diversity was significantly different than patients not on steroid therapy.

5.5 | Exclusive enteral nutrition

Exclusive enteral nutrition is effective and safe in treating paediatric CD.¹⁰⁴ Exclusive enteral nutrition induces paediatric CD remission possibly through multiple pathways, but recent studies have demonstrated that modulation of the gut microbiota may be one of the contributing factors.^{105,106}

In contrast with the other IBD treatments, the gut microbiota composition of paediatric CD patients receiving exclusive enteral nutrition moved significantly further away of the gut microbiota of healthy controls and the microbial diversity decreased.^{105,107,108} Guinet-Charpentier *et al* described a relative decrease of *Dialister*,

Blautia, unclassified Ruminococcaceae and *Coproccoccus* in the remission group on enteral therapy compared with the remission group not on enteral therapy. In the group treated with enteral nutrition, a relative decrease of the Proteobacteria phylum was observed, whereas *Alistipes* proportions significantly increased during the treatment.¹⁰⁶

In summary, literature on the role of the microbiome in predicting response to therapy in IBD is at an early stage, and quite scarce (only available for biological therapy).

Significant variance of gut microbiome between remitters and non-remitters, with specific genera showing differential relative abundance, was found. Overall, it concludes that patients with a more diverse microbiome composition at baseline, with a higher microbial diversity and lower baseline dysbiosis are associated with better response to biological treatment. A recurring fact in multiple studies is the relative higher abundance of SCFA-producing bacteria, such as *Bifidobacterium*, *E. rectale*, *Faecalibacterium prausnitzii* and *Roseburia*, or their bacterial metabolites (SCFAs, Branched chain amino acid biosynthesis and GAG degradation pathways), mucus-colonising bacteria and the relative increase of, for example, Enterobacteriaceae, *Veillonella* and *Fusobacterium* in non-responders. These findings strengthen the idea that the intestinal microbiome plays a role in initiation and propagation of intestinal inflammation, and that this process is further regulated by administration of biological therapy.

The bacterial groups that have been associated with treatment outcome overlap greatly with these associated with disease severity (see above), with some differences, although the pre-treatment samples did not significantly differ in baseline disease activity between the different treatment outcomes in the studied articles.

We need to point out that the bacterial groups that have been pointed forward in this review have not been independently replicated. This is not feasible at this moment in routine clinical practice.

Diet and lifestyle differences were not routinely assessed in all included articles, and consequently its effect on the gut microbiome cannot be excluded, although Aden *et al* and Kolho *et al* could not observe any difference between remitters and non-remitters.

There is no available literature on the predictive role of the gut microbiota on other IBD treatments besides biological therapy.

Microbiome genus frequency-based models and taxonomic composition based models of predicting response in IBD patients, combining clinical parameters with microbiota data and laboratory results, are able to accurately predict response with good accuracy.

We see that IBD treatments can have a possible effect on the gut microbiota composition.

Multiple studies describe a restoration of the microbial diversity when observing faecal microbiota profiles during biological therapy, shifting towards a healthy control microbiota profile also in non-remitters, in contrast with paediatric IBD patients on exclusive enteral nutrition, but data are conflicting. Ananthakrishnan *et al* and Doherty *et al* described several bacterial taxa decreasing/increasing during biological treatment with vedolizumab and ustekinumab, respectively, according to their therapeutic outcome, but no bacterial

groups have been described to deviate in the same direction for both remitters and non-remitters.

Exclusive enteral nutrition, thiopurines, mesalazine and steroids have been described to possibly influence intestinal composition in different manners.

Correlative studies are a good start but the current results cannot decipher causal relationships or mechanisms involved in microbiota modulation of treatment response or disease severity. Causal insights would, for example, need to come from germ-free, or gnotobiotic, mouse models with microbiomes that are derived from human donors, referred to as “humanized”. They enable to study the human microbiome in a model organism in which numerous variables can be controlled in a way that cannot be ethically or logistically achieved when studying humans. These models also allow to colonize with specific bacteria or consortia and therefore to determine whether specific IBD drugs can alter bacterial compositions or whether bacteria or bacterial groups can converse disease phenotypes, affect drug metabolism or serve as predictor.

Furthermore, the populations studied are small in sample size and patient groups are heterogeneous. Most of the published studies in patients treated with anti-TNF α therapy have no more than 10–15 samples for faecal microbiota analysed. Furthermore, there is no consensus on the definitions for response and remission, and none of the studies (so far) have taken in account prior drug exposure, which—according to recent data—is a very important factor contributing to response. Longitudinal follow-up samples and independent validation cohorts are also necessary in prediction research to prove the applicability of the identified biomarkers.

6 | CONCLUSION AND FUTURE PERSPECTIVES

Microbial biomarkers of disease activity and treatment efficacy have the potential to transform clinical practice and inform optimal and more personalized treatment strategies. Nevertheless, despite the massive advances in the microbiota field during the last decade, there are many obstacles before translating the findings into therapeutic applications.

At the moment, there is a lack of consistency between studies regarding which residents of the microbiota are the key players in IBD. The reasons for this include differences in methodology used and reference databases used for analysis. Another reason is the increasing awareness that microbiota composition is maybe less important than their function, which may change depending on the environmental conditions. Nevertheless, the impact of the gut microbiota on therapeutic responses in IBD is increasingly recognized, and the data in this review provide possible evidence that the gut microbiota may improve disease monitoring, and may modulate IBD treatment success, in the same manner as what has been described for chemotherapy and immune checkpoint blockade in cancer therapy.

One general observation is that higher baseline richness (without significant difference in baseline disease activity) and microbial

diversity (Table S1) are associated with better outcome and a less diverse or rich microbiota is associated with a more severe disease state.

Some caveat is needed as lifestyle habits (eg physical activity status, diet, smoking habits and living conditions) have not been included in most studies.

During an IBD flare, microbiota profiles from IBD patients tend to converge towards a more bare, homogenous dysbiotic profile, where commensal microorganisms become pathogenic or make place for more opportunistic bacteria.

Several bacteria have also been repeatedly associated with treatment response (*Faecalibacterium*, *Bifidobacteria*) or non-response (*Veillonella* and *Fusobacterium*) and disease activity, although reproducibility is still lagging behind.

Studies so far include only associations or correlations, and fail to demonstrate causal relationship between gut microbiota and treatment response or disease severity. The challenge of establishing causality is not new, but is critical. Cohort studies such as the GEM project, a large prospective study of healthy family members of CD patients, to identify pre-clinical disease biomarkers, may provide some of the answers. Microbial changes before and during the years of disease could provide clues how to counteract a non-permissive gut profile. We have learned from mouse studies that a deliberate alteration of the gut microbiota can induce disease or change the behaviour, with faecal microbiota transplantations in severe recurring *Clostridium difficile* infections as the most important evidence for this in humans with gastro-intestinal disorders.¹⁰⁹ Advances in genome editing may in the future enable the targeted deletion of microbial genes in clinical scenarios in which it is clear that treatment can be achieved with modification of a single process within the microbiome.¹¹⁰

In the coming years, even more biological agents and small molecules will be introduced in the clinic in IBD. Increasing therapeutic choices come with an increasing need to predict outcome. Therapeutic response is known to be multifactorial and influenced by multiple environmental, demographic, clinical and disease factors. Inflammation and microbiome composition are linked and intertwined with each other. There is growing belief that factors as diet, nutrition, stress, genetics, medication and lifestyle influence the gut ecosystem and thereby influence also drug response.

If we want to implement microbiome biomarkers, future studies should be designed to create a useful predictive microbial signal, index or model that meets all the requirement and, most off all, predicts disease activity or treatment efficacy/side effects, with better accuracy than the markers currently used. Including the faecal metabolome and detailed questionnaires on nutrition, stress levels and lifestyle before and during start of therapy, may be the way forward.

Finally, the lack of reproducibility of microbiome signatures might be solved in time by advances in the microbiome research field. Such recent improvements include improved quality control of sequencing errors,¹¹¹ and also the transition from characterising the microbiota as proportions to quantitative cell counting.⁷¹ Without quantitative data,

it is impossible to know if a particular bacterium is more abundant under specific conditions, especially in conditions such as IBD, where the microbial load is tightly linked to inflammation. Quantitative microbiota profiling could drastically improve detection of links between microbiota residents and quantitative health parameters, such as inflammation biomarkers. The continuing maturation of the microbiome research field will certainly facilitate the future identification of prediction biomarkers and improve their validation and reproducibility.

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AUTHORSHIP

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Author contributions: CC performed the research. CC, SVS, SV wrote the paper. NSST designed figures and corrected the paper. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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