PRIMARY NON-RESPONSE IN INFLAMMATORY BOWEL DISEASE, DEFINITION, POTENTIAL CAUSES, THERAPEUTIC DRUG MONITORING AND MICROBIOTA – A REVIEW

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Abstract. Tumor necrosis factors (TNF-α) are pro-inflammatory cytokines centrally involved in autoimmunity. Monoclonal antibodies against TNF-α are used to treat several autoimmune diseases including inflammatory bowel disease (IBD). The proportion of patients who experience primary non-response (PNR) to anti-TNF treatment is approximately 13–40%. Secondary loss of response (LOR) to anti-TNF agents happens in 23–46% of IBD patients leading to a drug discontinuation rate of 5–13%. A combination of factors including disease characteristics (e.g., phenotype, location, and severity), drug response (e.g., pharmacokinetics, pharmacodynamics, or immunogenicity), and treatment strategy (e.g., dosing regimen) has been associated with PNR and LOR. Therapeutic drug monitoring (TDM) relies on the measurement of serum concentrations of anti-TNF agents and anti-drug antibodies. TDM can be utilized to identify PNR and LOR and to assist clinicians in their decision-making. Additionally, TDM is used to optimize drug therapy (e.g., dose escalation) for patients who exhibit LOR. Recently, gut microbiota was believed to play a central role in immune regulation, and influence response to TNF-α antagonists. Microbial diversity for certain taxa can become a prognosis factor to monitor the response to treatment. In this article, we aim to review PNR and LOR, and discuss microbiota profiles associated with their occurrence.

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Abbreviations: TNF: tumor necrosis factors, IBD: inflammatory bowel disease, PNR: Primary non-response, LOR: secondary loss of response, TDM: therapeutic drug monitoring, UC: ulcerative colitis, CD: Crohn's disease, IFX: Infliximab, ADL: Adalimumab, CZP: Certolizumab, GOL: Golimumab, CRP: C-reactive protein, ADAs: antidrug antibodies, AZA: Azathioprine, SCFAs: short-chain fatty acids, SpA: spondyloarthritis, NSAID: nonsteroidal anti-inflammatory drugs

Background

Tumor necrosis factors (TNF-α) are pro-inflammatory cytokines produced by certain cell types, such as T-cells and macrophages (Ebert et al., 2008). The number of these cells is increased in the intestinal mucosa of patients with inflammatory bowel disease (IBD). Accordingly, these cells are used as therapeutic targets. TNF-α functions as a component of the intestinal mucosa-mediated defensive line against mucosal pathogens and destructive inflammation (Allendoerfer and Deepe, 1998). TNF-α antagonists are monoclonal antibody drugs that are considered a revolutionary treatment for IBD. It has been demonstrated that TNF-α antagonists contribute to improving life quality of IBD patients and limit the requirement for surgeries and hospitalizations (Wang et al., 2019). Treatment guidelines encourage early utilization of TNF-α antagonists for IBD patients, particularly for patients who are refractory to other classes of medications and have been found to exhibit high-risk features at baseline (Gomollón et al., 2016). TNF-α antagonists are approved for the induction and maintenance of remission for both types of IBD, e.g., ulcerative colitis (UC) and Crohn's disease (CD) (Ha et al., 2012).

In cases of moderate-to-severe CD, intensive treatment regimens incorporating TNF-α antagonists, such as infliximab (IFX), adalimumab (ADL) and certolizumab (CZP) have been proven to be effective and can lead to clinical remission and mucosal healing (Hazlewood et al., 2015). IFX, ADL and GOL (golimumab) have been approved for the induction and maintenance of remission in UC. Despite this, the use of TNF-α antagonists is limited due to the cost and possibility of unpredictable side-effects, including infusion reactions, infections and lymphoma (Singh et al., 2011; Ben-Horin and Chowers, 2011). A small percentage (5%) of IBD patients has been recorded to experience adverse drug reactions with severity ranging from simple skin rashes to anaphylactic reactions. It has been estimated that 10-30% of patients treated with TNF-α antagonists may not respond, and these patients are referred to as primary non-responders (PNRs). Additionally, 23-46% of patients may experience loss of response (LOR) over time, and this situation is accordingly referred to as secondary LOR (Roda et al., 2016). While LOR is mainly attributed to pharmacokinetic derangements, the precise etiologies underlying PNR are unknown (Ebert et al., 2008; Ben-Horin and Chowers, 2011; Billioud et al., 2011). The gut microbiota is recently thought to play a central role in immune regulation, and the accumulating literature on this process suggests that it also influences response to TNF-α antagonists (Zhang et al., 2015; Rajca et al., 2014).

Mechanisms underlying PNR can be attributed to pharmacokinetic failure (Rocha et al.), pharmacodynamic failure (Ainsworth et al., 2008) and immunogenicity failure (Rojas et al., 2005). The causes of PNR to anti-TNF therapy are unknown, however, the possible factors contributing to PNR can be classified into four categories, patient-related factors, microbiome-related factors, disease-related factors and treatment-related factors (Ding et al., 2016). PNR can be often managed through optimization of dosing regimen (Hanauer et al., 2002) and combination therapy (Colombel et al., 2010; Coutinho et al.,

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1995). Therapeutic drug monitoring (TDM) play a fundamental role to determine the appropriate assessment time for PNR occurs which at weeks 12 to 14 following induction (Papamichael et al., 2014; Cornillie et al., 2014). The human gut contains more than 100 trillion different microbial organisms, including more than 1000 species of bacteria, viruses, fungi and protozoa, collectively referred to as the microbiome (Honda and Littman, 2012). Four phyla are predominant and represent more than 99% of intestinal bacteria, which are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (Eckburg et al., 2005; Ley et al., 2008). The *Firmicutes* and *Bacteroidetes* phyla represent the main commensal microbiota in healthy subjects, while *Proteobacteria* and *Actinobacteria* are significantly higher in IBD patientsqw (Sheehan et al., 2015; Andoh, 2016). Fecal microbiota transplantation (FMT) aims to recover the gut microbial level in patients via transferring fecal suspension from a healthy donor (Wang et al., 2017). In this article, we aim to explore PNR and LOR with a focus on the underlying causes of PNR and the possible involvement of gut microbiota.

Primary non-response (PNR) definition

A precise definition of PNR has not been determined, however, the accepted definition of PNR in connection with the use of anti-TNF-α drugs is failure to achieve clinical remission following the induction therapy period (Sprakes et al., 2012). It has been demonstrated that anti-TNF antagonists such as CZP, ADL and IFX are efficient for eliciting prompt remission and to prevent relapse in IBD. Despite the known efficacy of these drugs, it is recommended that clinicians estimate the improvement in the clinical signs after 8, 12 and 14 weeks, respectively, following the initial infusions/injections with these drugs in PNR patients (Hanauer et al., 2002; Sands et al., 2004). Data from clinical trials and clinical practice differ in regard to the incidence of PNR, which ranges from 10 to 30% (Sprakes et al., 2012; Hanauer et al., 2002; Ford et al., 2011).

Several factors may contribute to the risk of PNR, including a disease duration of longer than 2 years, small bowel involvement, smoking, elevated C-reactive protein (CRP) and genetic mutations in apoptosis-related genes, such as FAS-L and caspase-9 (Ben-Horin et al., 2014). PNR can be minimized by optimization of the initially selected dosing regimens, by increasing the dose or reducing the intervals between doses, and by combining TNF-α antagonists with immunosuppressants, such as thiopurines or methotrexate (*Table 1*) (Ding et al., 2016; Roda et al., 2016). The latter approach is supported by data from several clinical trials. PNR is typically managed by switching to a different type of TNF-α antagonist that could be beneficial. However, several studies have demonstrated that the treatment outcome following a switch to a second anti-TNF antagonist in PNR patients is still poor with a response rate of approximately 50–65% (Allez et al., 2010). Switching to an out-of-class medication that acts through different mechanisms may provide a worthwhile resolution to this problem (Sands et al., 2014). The proportion of PNR can differ among clinical trials (36–40%) and according to clinical practice (13–33%) (Ford et al., 2011).

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Table 1. *A comparison between primary non-response (PNR) and secondary loss of response (LOR) (Ding et al., 2016; Roda et al., 2016)*

	Primary non-response (PNR)	**Secondary loss of response (LOR)**
Definition	Remission does not occur during the induction of therapy period and clinical signs and symptoms are continuous (no healing)	The patients who respond to the initial induction of therapy but subsequently suffer from clinical relapse and lack of remission albeit maintenance of therapy
Percentage of those who do not respond	10-30%	23-46%
Incidence	Differs between clinical trial and clinical practice from 10–20% to 13–30%	Its incidence is 13% for Infliximab (IFX) and 24% for Adalimumab (ADA)
Risk factors	- Disease longer than 2 years - Small bowel involvement - Smoking - C reactive protein - Genetic mutations such as FAS-L and caspase-9 in the apoptosis related genes	- Formation of antibodies against TNFα antagonists (immunogenicity) - Use of episodic TNFα antagonists in 5–13% of patients
Management	- Optimization of the dosing regimen - Combination therapy	Use of concomitant immunomodulators with Anti -TNFα antagonists
Therapeutic options	- Switching to another anti- TNF could be beneficial - Switch out of the therapeutic groups that are characterized by other working mechanisms	- Change to another TNFα antagonist agent was associated with a complete or partial response in 92% of patients - Switching within a therapeutic class to another anti-TNF agent may restore clinical response
Strategy	- Dose escalation based on the pharmacokinetic - Therapeutic drug monitoring (TDM)	Therapeutic drug monitoring (TDM)

Proposed mechanisms underlying PNR

Three mechanisms that could explain PNR to TNF-α antagonists are presented in *Figure 1*.

Pharmacokinetic failure

This phenomenon occurs when suboptimal levels of TNF-α antagonists circulate either because of suboptimal dosing or interaction with anti-drug antibodies (Rocha et al.) that leads to accelerated drug clearance (non-immune) via tissues or through the systemic circulation. The three fundamental mechanisms implicated in pharmacokinetic failure include:

i. Proteolytic catabolism that occurs in the reticuloendothelial system due to the ability of monoclonal antibodies to bind to Fc gamma receptors (Fc, or Fragment/crystallizable, is a surface protein, and the term is derived from the proteins' specificity to bind a part of an antibody known as the Fc region.
ii. Degradation that occurs in lysosomes as a result of interaction with membrane-bound TNF (Keizer et al., 2010; Ordás et al., 2012).

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iii. Ulcerated mucosa that leads to clearance and drug loss through the mucosal membrane as a result of non-immune clearance, ultimately resulting in considerable loss of protein and electrolytes in addition to loss of drug (Brandse et al., 2015).

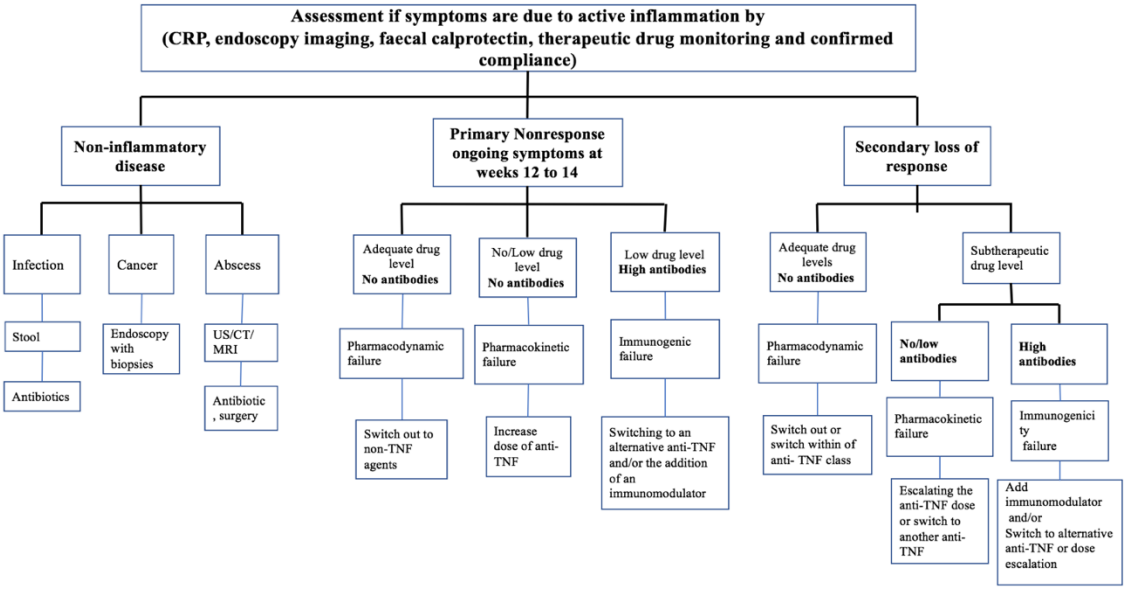

Figure 1. *Management of primary nonresponse and secondary loss of response. Taken from (Ding et al., 2016; Roda et al., 2016)*

Pharmacodynamic failure

This type of failure occurs when no improvement in clinical symptoms occurs despite the presence of adequate circulating drug and absence of Antidrug antibodies (ADAs) (Ainsworth et al., 2008). The most favorable alternative avenue would be to switch to an out-of-class medication, such as a leukocyte trafficking inhibitor or an anti-cytokine (Ding et al., 2016).

Immunogenicity failure

This scenario is characterized by a lack of improvement in symptoms in the presence of low circulating serum TNF-α antagonists and high levels of ADAs. One of the strongest factors linked to non-response is the formation of ADAs against anti-TNFα antagonists. Antibodies possess the ability to interfere with TNF receptors and to accelerate the clearance of the drug through the reticuloendothelial system. Low levels of ADAs have been implicated in effectively achieving remission (Rojas et al., 2005). Neutralizing and non-neutralizing antibodies and low drug concentrations have been reported in up to 83% of PNRs (Echarri et al., 2014). Additionally, the effective induction of remission in PNRs by using a second TNF-α antagonist occurs in only 50% of IBD patients (Gisbert et al., 2015). The perfect option is to switch to an alternative anti-TNF or to incorporate the use of an immunomodulator. Following a drug switch, therapeutic drug monitoring should be repeated to determine if antibody disappearance has occurred (Ding et al., 2016).

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Potential causes of PNR to anti-TNF therapy

The causes of PNR to anti-TNF therapy are unknown; however, the possible factors contributing to PNR can be classified into four categories (see *Fig. 2*).

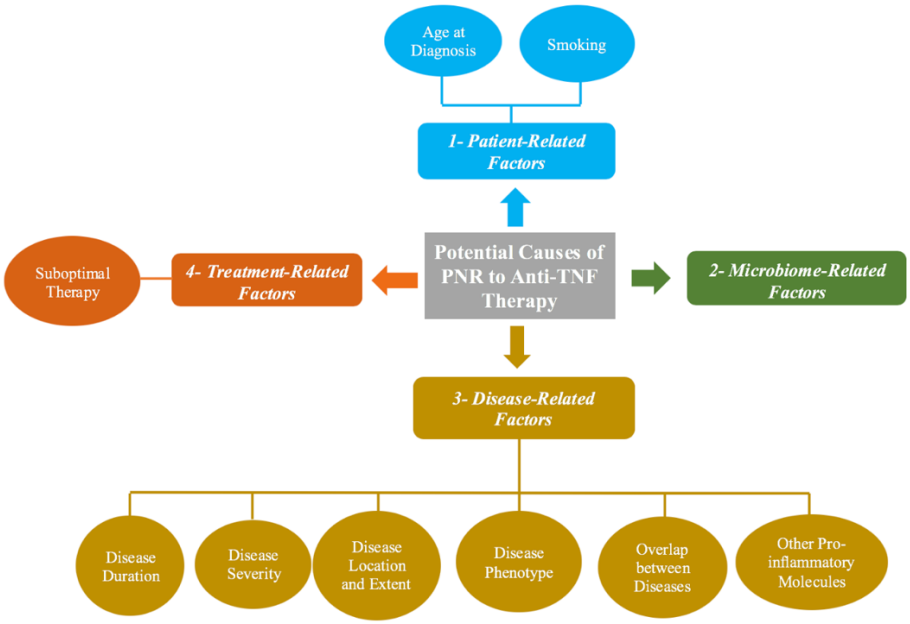

Figure 2*. Potential causes of PNR to anti-TNF therapy*

Patient-related factors

Factors like gender, lack of concomitant immunosuppression, age, prolonged duration of disease, smoking, CD phenotype, and disease not limited to the colon may contribute to the response to anti-TNF α agents (Danese et al., 2011). The two most patient-related factors include:

Age at diagnosis

The connection between age at the time of diagnosis and PNR is controversial (Juillerat et al., 2014), although diagnosis at an early age (less than 17 years) is often associated with poor outcome (Grover et al., 2014). Interestingly, younger patients tend to respond better to anti-TNF therapy in comparison with older patients (Vermeire et al., 2002).

Smoking

Smoking is an environmental factor that likely plays a role in reducing patient responsiveness to anti-TNF agents. A relationship between smoking and PNR has been previously reported. Approximately 30% of patients who are smokers are non-responsive to IFX at week 4 (Arnott et al., 2003). Smoking has been found to decrease the influence of anti-TNF drugs and to increase the likelihood of non-response (Cohen et al., 2011; Chaparro et al., 2011). According to a study performed on 221 ADL-treated patients,

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21.2% of the patients were smoking at the time of induction (OR 0.52, P = 0.049) (Ding et al., 2016; Kiss et al., 2011). Additionally, PRECISE-3 data indicated that smokers had more active disease compared to non-smokers (HR: 1.404; 95% CI: 1.09–1.77; P = 0.007) (Sandborn et al., 2015).

Microbiome-related factors

The exact role of the gut microbiota in PNR is not well understood. Several studies have observed no significant difference in the gut microbiota composition before and after treatment with TNF-antagonists (Zhang et al., 2015). In contrast, results published by Bazin et al. (2018) indicated that gut microbial composition could be used as a biomarker that is predictive of clinical response to anti-TNF treatments. Additionally, microbial diversity in the presence or absence of particular taxa has been used as a prognostic factor to monitor the response to treatment or the presence of several diseases, including colorectal cancer (Gagnière et al., 2016; Bazin et al., 2018). It has been found that in some diseases, such as melanoma, particular microbiota species, can be used as biomarkers to determine the correlation between the colitis and resistance to immunotherapy (Dubin et al., 2016).

Recent study compared the microbial composition among anti-TNF therapy-treated UC patients; responders showed increase of the concentrations of *Faecalibacterium prausnitzii* and decrease of the rate of dysbiosis compared with non-responders. Furthermore, both responders and non-responders had a featured mucosal antimicrobial peptides expression patterns (Magnusson et al., 2016). In addition, another recent study showed that in the case of discontinuation of using anti-TNF-α treatment, low abundance of *F. prausnitzii* can be used as a biomarker to predict the early incidence of Crohn's disease (Rajca et al., 2014). In rheumatology, dysbiosis in the oral and gut microbiota of rheumatoid arthritis patients had been monitored. This dysbiosis can be partly treated by using disease-modifying antirheumatic drugs (DMARD) (Zhang et al., 2015). However, the influence of rheumatoid arthritis was moderate on the gut microbiota compared to the oral microbiota. Moreover, decrease of risk factor and low concentration of *Holdemania filiformis* and *Bacteroides* species have been observed in the responder patients after therapy (Zhang et al., 2015).

Disease-related factors

A range of disease-related factors has been associated with PNR. They are the following:

Disease duration

According to several studies, patients with shorter disease durations (< 2 years) exhibit better responses and higher long-term remission rates compared to those of patients who have had the disease for more than 2 years. For instance, the Crohn's Trial of the Fully Human Antibody Adalimumab for Remission Maintenance (CHARM) study was performed to determine the rates of response and remission to ADL. Assessments were performed at week 26 to evaluate the impact of disease duration on response and remission rates. More patients with a short disease duration experienced response compared to patients with a disease duration of > 2 years or > 5 years (56%, 35% and 37%, respectively) (Colombel et al., 2007). Similar results were observed in the PRECISE 2 study that evaluated remission and response to certolizumab pegol (CZP) in CD at week 26. Of the CD patients treated with CZP, 62% exhibited PNR

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(p = 0.02) (Schreiber et al., 2010). Additionally, greater rates of response and remission were observed in CD patients with disease duration of < 2 years compared to those of patients with disease duration of ≥ 5 years (Reinisch et al., 2009; Colombel et al., 2015).

Disease severity

Several studies suggest that disease severity is one of the main factors that contribute to non-response (Castro-Laria et al., 2016; Reinisch et al., 2011). The efficacy of anti-TNF treatment has been observed to be lower in severely inflamed tissue. This is likely due to hastening of non-immune drug clearance (Fasanmade et al., 2009, 2011). PNR could also be attributed to the use of inadequate induction dosages. It has been proposed that fecal loss of anti-TNF drugs via ulcerated, denuded mucosa contributes to PNR (Brandse et al., 2015).

Disease location and extent

Although some studies suggest the presence of a correlation between localized ileal stricture disease and PNR, much of the data regarding this correlation remains conflicting (Louis et al., 2007). One study proposed ileal resection as an effective treatment for localized ileal stricture disease, but a separate study suggested that the localization of disease did not directly affect the response rate. The study compared two categories of patients who were treated with anti-TNF therapy, e.g., patients with isolated ileal stricture disease and patients with stricture disease at an unspecified location. The results indicated that both patient categories required surgery at the same rate (Moran et al., 2014).

Disease phenotype

An association between disease phenotype and response to anti-TNF therapies has been suggested in several studies. In Kiss et al. (Kiss et al., 2011; Ding et al., 2016), which included 201 CD patients treated with ADL, at week 52, PNR with continued clinical remission had been observed in patients who have active luminal disease (OR: 3.89; 95% CI: 1.43–10.6; P = 0.008). Moreover, at week 12, it had been noticed that the presence of two pathological phenotypes, luminal and fistulizing in CD patients led to decrease of remission rates. Otherwise, this rate was sort of high in CD patients with only luminal phenotype (42.5% vs. 56.3%, P = 0.06).

Overlap between diseases

An overlap between inflammatory diseases such as Spondylarthritis (SpA) and IBD is possible. For instance, 5–10% of SpA patients may have concomitant IBD, while up to 30% of IBD patients may also experience inflammatory arthritis (Bazin et al., 2018). Additionally, 60% of SpA patients have microscopic gut inflammation (Lin et al., 2014; Van Praet et al., 2012). These overlaps may often explain resistance to treatment with TNF antagonists.

Other pro-inflammatory molecules

Theoretically, IBD patients characterized as PNR may not benefit from switching between IFX and ADL since both drugs possess the same chemical structure and function (Dassopoulos, 2005). Accordingly, a lack of response to anti-TNF agents could be due to specific disease characteristics, with dose intensification would not achieve the required

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result. A potential explanation for this is that pro-inflammatory molecules other than TNF-α may be responsible for the pathogenesis of the disease (Gisbert et al., 2015).

Treatment-related factors

A range of treatment-related factors is associated with PNR. They are the following:

Suboptimal therapy

The common indicators of suboptimal therapy are dose escalation of anti-TNF and discontinuation of treatment. Among the anti-TNF patients, 25.8% of UC patients required dose-escalation and 19.2% of CD patients required increased doses. The underlying reason for these therapeutic alterations is the worsening of clinical signs and symptoms (94.2% UC and 94.5% CD). Among UC patients, the cause of discontinued initial anti-TNF therapy was the appearance of negative clinical symptoms (45.6%) or the occurrence of an adverse reaction (23.2%). Additionally, 49.5% of discontinued UC patients were switched to an alternate anti-TNF therapy. Among CD patients, the cause of discontinued initial anti-TNF therapy was uncontrolled symptoms (36.3%) or an adverse reaction (27.4%). Additionally, 62.7% of discontinued CD patients were switched to another anti-TNF therapy (Lindsay et al., 2017).

Rescue therapeutic strategies in cases of PNR

PNR is often managed through the following two strategies:

Optimization of dosing regimen

Data derived from CLASSIC 1 (Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in Crohn's Disease) demonstrated that a higher dosage of ADL could achieve better remission rates at week 4 of treatment compared to that of lower dosages. Similarly, data from the PRECISE-2 (Pegylated Antibody Fragment Evaluation in Crohn's Disease: Safety and Efficacy) and ACCENT-1 (A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen) trials demonstrated that higher dosages of CZP and IFX during the induction period are associated with a lower risk of PNR (Hanauer et al., 2002).

Combination therapy

Results obtained from the SONIC (Study of Biologic and Immunomodulator Naïve Patients in Crohn's disease) suggested that AZA (Azathioprine) exerts an additive influence on mucosal healing at week 26 when combined with IFX. Accordingly, combining anti-TNF drugs with immunosuppressive therapy appears to enhance drug efficacy and can theoretically help to prevent PNR (Colombel et al., 2010; Coutinho et al., 1995).

The role of therapeutic drug monitoring (TDM) in PNR

The appropriate assessment time for PNR occurs at weeks 12 to 14 following induction therapy (Papamichael et al., 2014; Cornillie et al., 2014). In week 4, 5 mg/mL serum concentration of adalimumab was used as an indicator to identify the risk of antibody formation. In a study on adalimumab-treated CD patients (n = 168), mucosal healing is

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associated with a trough level, and this can be utilized to predict clinical response. The median concentrations of ADL in serum were 8.6 lg/mL (interquartile range (IQR): 6.5–10.8) at week 2 and 5.3 lg/mL (IQR, 2.8–10.9) at week 4. At week 4, a comparison was performed between two types of patients, including those who received 80/40 mg and those who received 160/80 mg as a loading dose. The second patient group exhibited higher adalimumab serum concentrations (3.6 vs. 11.6 lg/mL; $P < 0.0001$) and possessed a lower incidence of PRN "as needed" (odds ratio [OR]: 0.02; 95% CI: 0.003–0.2; $P < 0.0001$) (Karmiris et al., 2009; Ding et al., 2016). A serum trough concentration of < 5 mg/mL has been associated with an increased future risk of the formation of antibodies specific to ADL (HR: 25.12; 95% CI: 5.64–111.91; $P = 0.0002$) (Baert et al., 2016).

A prospective study that examined serum drug concentrations of 32 CD patients treated with IFX (n = 15) and ADL (n = 17) at week 14 demonstrated that responders possessed a higher trough concentration than that of non-responders (FX [5.60 lg/mL]. ADL was compared to non-responders using the Harvey–Bradshaw Index, C-reactive protein (CRP) or fecal calprotectin concentration [IFX 0.032 lg/mL and ADL 2.62 lg/mL; $P = 0.01$]) (Echarri et al., 2014). At week 6, high trough concentrations of IFX (> 3 lg/mL) and ADL (> 4.5 lg/mL) were used, and > 90% remission and response rates were achieved. Additionally, sustained anti-drug antibody levels were observed in 26% of the IFX-treated patients and in 0% of the ADL-treated patients. In general, it has been suggested that the observation of adequate anti-TNF concentrations at weeks 4 to 6 is highly predictive of response to anti-TNF therapy. At week 14, a low anti-TNF drug concentration and the occurrence of antibody formation can predict primary non-response (Ding et al., 2016).

Proactive and reactive therapeutic drug monitoring

Proactive TDM is applied during remission. The aim of this approach is to modify the dose of IFX depending on individual pharmacokinetics and pharmacodynamics to avoid sub-therapeutic dosing and the risk of failure or to minimize the intensity of the therapy to reduce the financial costs associated with supra-therapeutic dosing. In contrast, reactive TDM is applied as a result of treatment failure despite the previous use of IFX therapy to achieve a successful outcome. This approach depends on pharmacokinetics and pharmacodynamic in response to IFX intensification, change to another TNF inhibitor, or the use of a new biologic drug class (Steenholdt, 2018).

Recent data suggest that proactive TDM of IFX leads to successful therapeutic outcomes in IBD. Despite this, the clinical benefits of proactive infliximab have not been confirmed after first reactive testing. A retrospective cohort study was carried out from September 2006 to January 2015 on IBD patients who underwent to maintenance IFX treatment and received an initial reactive testing (Papamichael et al., 2018). The purpose of this study was to compare outcomes at long-term between proactive infliximab monitoring after reactive testing and just reactive testing in IBD patients. Patients were divided into two groups; Group A represented a proactive infliximab monitoring after reactive testing while Group B represented a reactive testing alone. Treatment failure was defined as drug discontinuation due to either LOR or the occurrence of a serious adverse event. The total number of IBD patients was 102 (n = 70, 69% with CD; Group A, n = 33 and Group B, n = 69) were followed for a median of 2.7 years (interquartile range [IQR], 1.4–3.8 years). Multiple Cox regression analysis determined that patients who had proactive following reactive TDM were independently associated with less treatment

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failure (hazard ratio [HR] 0.15; 95% confidence interval [CI] 0.05–0.51; P = 0.002) and fewer IBD-related hospitalizations [HR: 0.18; 95% CI 0.05–0.99; P = 0.007]. Conclusion of this study was the proactive infliximab monitoring following reactive testing led to better drug stability and decrease of hospitalizations among IBD patients compared to reactive testing alone (Papamichael et al., 2018).

A multicenter retrospective cohort study was performed from June 2006 until December 2015 on IBD patients who underwent to maintenance adalimumab therapy (Papamichael et al., 2019). The study aimed to evaluate long-term the outcomes between IBD patients who had at least one proactive TDM of ADL with standard of care and/or reactive TDM. Treatment failure was defined as drug discontinuation due to secondary LOR, the occurrence of a serious adverse event, or the need for IBD-related surgery. The total number of IBD patients was 382 (Crohn's disease, n = 311, 81%) received at least one proactive TDM (n = 53) or the standard of care (empirical dose escalation, n = 279; reactive TDM, n = 50). Patients were followed for a median of 3.1 years (interquartile range, 1.4-4.8 years). Multiple Cox regression analyses demonstrated that obtaining at least one proactive TDM led to decrease the risk of treatment failure (hazard ratio [HR]: 0.4; 95% confidence interval [CI]: 0.2–0.9; p = 0.022). The study provided the foremost evidence that reducing of risk of treatment failure may be attributed to proactive TDM of ADL compared with standard of care (Papamichael et al., 2019).

Microbiota profiles and primary non-response to anti-TNF agents

The human gut has more than 100 trillion various microbial organisms, including more than 1000 species of bacteria, viruses, fungi and protozoa, collectively referred to as the microbiome (Honda and Littman, 2012). Four phyla are predominant and represent more than 99% of intestinal bacteria, which are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (Eckburg et al., 2005; Ley et al., 2008). The *Firmicutes* and *Bacteroidetes* phyla represent the main commensal microbiota in healthy subjects, while *Proteobacteria* and *Actinobacteria* are significantly higher in IBD patients (*Figs. 3* and *4;* Sheehan et al., 2015; Andoh, 2016).

Functional composition of gut microbiota in IBD patients

Taxa of *Faecalibacterium*, *Odoribacter*, *Leuconostocaceae*, *Phascolarctobacterium* and *Roseburia* provide short-chain fatty acids (SCFAs) through a process that involves the fermentation of undigested carbohydrates. SCFAs are responsible for the regulation of trans-epithelial transport, colonocyte proliferation and differentiation, mucosal inflammation, intestinal motility, and barrier function (Smith et al., 2013; Peng et al., 2009). The concentration of SCFAs is significantly reduced in IBD patients, and this is likely a result of a decrease in the bacteria that produce them. *Bifidobacterium* synthesizes vitamins such as vitamin K and the water-soluble B vitamins (LeBlanc et al., 2011). At the functional metagenomic level, amino acid synthesis required for the production of these vitamins is decreased and amino acid transporter genes are increased due to an increase in auxotrophic and pathobiont bacteria (Ahuja, 2015). Increased glutathione and riboflavin metabolism and increased toxin secretion are associated with an increase in sulphate-reducing bacteria, such as *Desulfovibrio* (Ahuja, 2015; Erickson et al., 2012).

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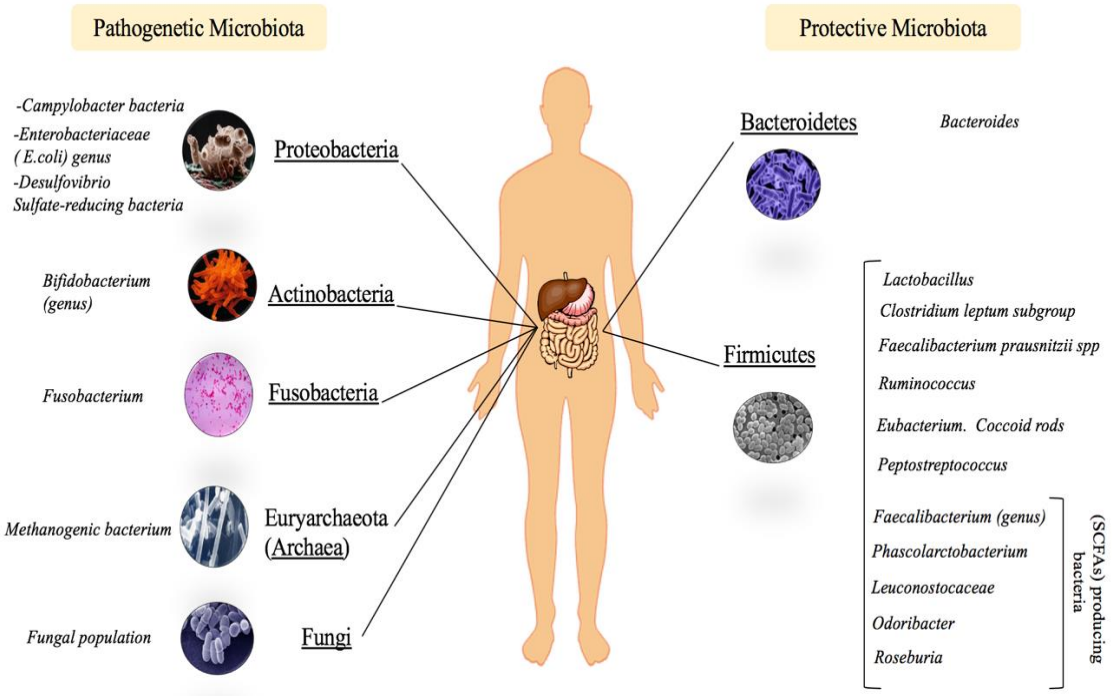

Figure 3. *Composition of gut microbiota (pathogenic and protective)*

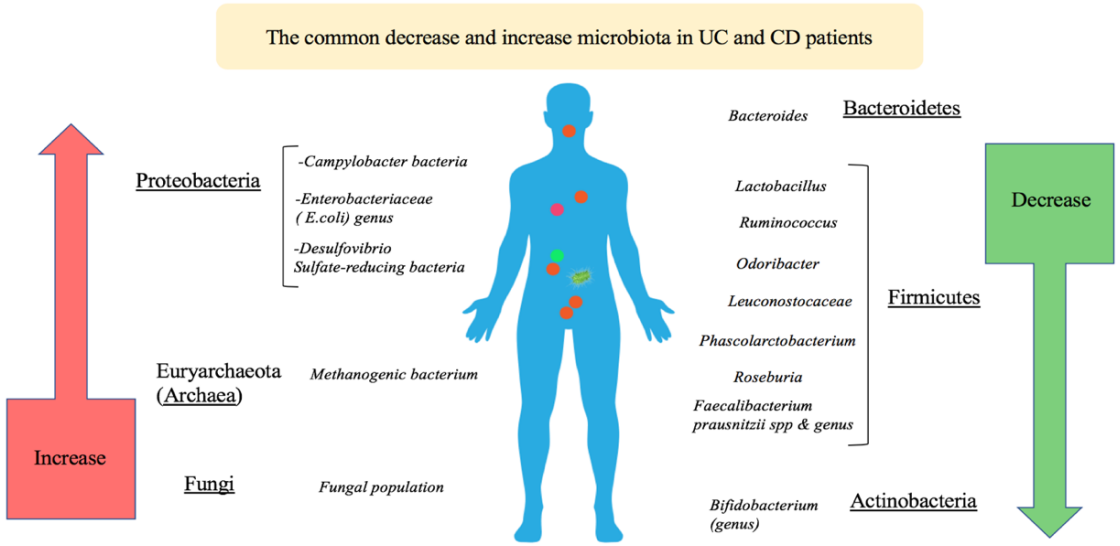

Figure 4. *The common decrease and increase of microbiota in UC and CD patients*

The clinical response to therapy is highly dependent upon the quantity and quality of bacterial taxa and upon any changes in bacterial taxa that occur in response to treatment. Accordingly, patients with few changes in their taxa in response to treatment typically exhibit improved drug responses. In contrast, patients who exhibit drastic changes in many bacterial taxa following treatment are believed to exhibit poorer drug responses. This hypothesis suggests that patients possessing an unstable gut microbial composition

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may possess a higher risk for anti-TNF-α treatment failure (Bazin et al., 2018). Additionally, TNF-α inhibitors may affect the composition of the gut microbiota, either directly or indirectly. TNF-α inhibitors are characterized by their ability to cure and downregulate inflammation in the infected gastrointestinal tract mucosa. These drugs also seek to repair wounded digestive epithelium and rebalance the composition of mucosal microbiota. These functions of TNF-α inhibitors may indirectly affect the microbiota composition of the gut (Baert et al., 1999).

Bazin et al. (2018) demonstrated that the gut microbial composition could be used as a predictive biomarker for clinical response to anti-TNF. Interestingly, such a predictive characteristic of anti-TNF inhibitors has been confirmed in several studies examining a number of different diseases. Additionally, microbial diversity in the presence or absence of particular taxa has been used as a prognostic factor to monitor diseases such as colorectal cancer or response to treatment (Bazin et al., 2018). For instance, an increase in the quantity of cyclomodulin-producing *E. coli*, *enterotoxigenic Bacteroides fragilis*, and *Fusobacterium nucleatum* was observed in cases of advanced colorectal cancer (Gagnière et al., 2016). Similarly, alteration in the microbiota composition has been used to explain resistance to immunotherapy in melanomas. It has also been postulated that some microbiota species possess the capacity to modify and improve the effects of therapy (Dubin et al., 2016; Routy et al., 2018).

A recent study demonstrated a decrease in dysbiosis and an increase in the quantity of *Faecalibacterium prausnitzii* exists in patients with UC who respond to anti-TNF therapy when compared to these factors in non-responders (Magnusson et al., 2016). Additionally, the results of this study demonstrated that responders and non-responders exhibit distinct expression patterns of mucosal antimicrobial peptides. Their findings also suggested that a relationship exists between decreased concentrations of *F. prausnitzii* and clinical relapse in CD patients treated with anti-TNF-α therapy (Rajca et al., 2014).

For the treatment of inflammatory diseases such as IBD and Spondyloarthritis (SpA), a therapeutic revolution occurred after the identification of tumor necrosis factor-alpha (TNF-α) antagonists, and this was particularly true for patients who had previously failed to respond to NSAID and conventional DMARDs (Sedger and McDermott, 2014; Ward et al., 2016). A number of studies have demonstrated that changes in the composition of the gut and mouth microbiome occur following the onset of several diseases. Subsequently, altered gut and mouth microbiomes are again partially modified in response to treatment, and these alterations can potentially predict response to treatment (Zhang et al., 2015; Phillips, 2015).

The effect of treatment on the gut microbiome is considered moderate when compared to these effects on the oral microbiome. It has been suggested that a higher probability of response occurs in patients who possess a significant number of virulence factors prior to therapy (Phillips, 2015).

Variations in the gut microbiota signature

In 2015, Zhang et al. (2015) performed an experiment on rheumatoid arthritis patients. They reported that differences observed in the gut microbiota composition before and after non-biologic DMARDs treatment were not significant. Additionally, Busquets et al. (2015) demonstrated that treatment with a TNF-α inhibitor such as ADL can affect the gut microbial composition of CD patients via recovery of *Firmicutes*, *Bacteroides*, and *Actinobacteria* phyla and a decrease in E. coli during treatment. This result was not observed in the study by Bazin et al., which may be owing to the different patient

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population that was studied and the therapies that were used (Bazin et al., 2018). Coyte et al. (2015) reported that an unstable microbiota composition was observed in non-responders over time. Considerable alteration in the microbiota composition has been linked to inflammatory diseases such as neurodevelopmental disorders and IBD, but it has not been associated with Spondyloarthritis. Magnusson et al. (2016) observed that UC patients who possess the capacity to respond to the induction of anti-TNF-α therapy exhibited a high abundance of *F. prausnitzii* compared to that of non-responder patients. A significant proportion of *Lactobacillus delbrueckii* has been previously observed in responder patients. These bacteria possess the ability to ferment kefir and are used as probiotics for IBD therapy (Rocha et al., 2014).

Several studies that were performed on humans have confirmed that the *Bacteroidetes* population is greater than that of *Firmicutes* in IBD patients compared to healthy controls (Wright et al., 2015). However, conflicting data were reported in a study by Rooks et al. (2014) compared mice that were treated with anti-TNF antibodies post colitis to mice that were treated with an antibiotic; *Firmicutes* populations were increased and the proportions of *Bacteroidetes* were decreased in the mice treated with anti-TNF antibodies. These contrasting results highlight the finding that microbial responses are different after anti-TNF treatment. Based on this, patient responses to anti-TNF treatment may also differ (Chiodini et al., 2013). *Firmicutes* is considered the most common phylum that is restored in CD patients treated with ADL. E. coli levels are also often increased in CD patients (Busquets et al., 2015). Bazin et al. also observed that the majority of non-responders exhibited changes in the order *Bacteroides* quantity, where two patients exhibited a decrease in these bacteria and five patients exhibited an increase. Responders, however, did not exhibit any changes in the *Bacteroides* order. This illustrates that the proportion of the order Bacteroides differs significantly for IBD conditions. The relationship between microbiota composition clusters and clinical response has been demonstrated without the presumption of causality. Fecal microbiota signatures could be used to predict clinical response to anti-TNF-α therapy, particularly in the absence of reliable biomarkers (Bazin et al., 2018). A balanced diversity in the composition of symbiotic microbiota, such as bacteria, fungi, and viruses may be used to predict a positive outcome to treatment (Ciccia et al., 2016).

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) aims to recover the gut microbial level in patients via transferring fecal suspension from a healthy donor. FMT is associated with recurrent *Clostridium difficile* infection (CDI). CDI is an appropriate situation for FMT, as it refers to gastrointestinal dysbiosis with *Clostridium difficile* overgrowth (Cohen et al., 2010). A study cohort from Shanghai Children's Hospital, China, was used to investigate the influence of IFX on the composition and function of the fecal microbiota of CD patients and healthy controls (CD [n = 11], healthy control [n = 16], all fecal samples [n = 48], CD patient samples [n = 32], baseline [n = 8], various times during IFX therapy [n = 24], healthy individuals [n = 16]). Prior to IFX therapy in pediatric CD patients, a lower biodiversity in fecal microbiome composition, an increase in *Enterococcus,* and a decrease in SCFA-producing bacteria including *Anaerostipes*, *Blautia*, *Coprococcus*, *Faecalibacterium*, *Lachnospira*, *Odoribacter*, *Roseburia*, *Ruminococcus*, and *Sutterella*, were observed. Additionally, alterations in metabolic functions of the gut microbes in CD patients were noted. In post-IFX samples, IFX treatment restored the gut microbiota to a normal state in pediatric CD patients, and the

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abundance of SCFA-producing bacteria (the genera *Blautia*, *Faecalibacterium*, *Odoribacte*r, and *Sutterella*) was associated with sustained therapeutic response. The gut microbiota were also improved in terms of richness and diversity. During IFX treatment, levels of *Enterococcaceae*, *Planococcaceae*, and *Streptococcaceae* were reduced in pediatric CD patients. In contrast, *Coprococcus*, *Lachnospira*, *Roseburia*, and *Ruminococcus* levels were elevated in the CD patients after treatment with IFX; however, their increases were unstable (Wang et al., 2017).

A study that was performed on adult CD patients (n = 33) aimed to identify alterations in the gut microbiota after IFX withdrawal. In this study, Rajca et al. (2014) noticed that CD patients exhibiting long-term remission possessed higher concentrations of *Firmicutes* compared to that of the relapsed CD patients. Additionally, relapsed CD patients possessed low levels of *F. prausnitzii* and *Bacteroides* during the year prior to IFX withdrawal.

Conclusion

PNR and LOR are important challenges faced by clinicians who treat patients with IBD. Although the precise cause of PNR is not well characterized, the most acceptable reason for LOR to TNF antagonists is immunogenicity that leads to the development of ADAs, which ultimately neutralize the drug or hasten its clearance. Therapeutic drug monitoring (TDM) is useful for aiding appropriate therapeutic decisions in cases of treatment failure. Through the use of TDM, clinicians can choose among dose intensification, the addition of an immunomodulator, or switching between classes of drugs. Future research should focus on the underlying mechanisms responsible for the development of PNR and on strategies to overcome LOR. The exact role that the gut microbiota plays in the process of treatment failure remains poorly understood. Microbial diversity in the presence or absence of particular taxa can be used as a prognosis factor to monitor the response to treatment. Strongly recommended increasing the prospective clinical trials to study the modification of the gut microbiota composition and determine which microbe(s) are responsible for primary non-response or loss of response and thereby used as a biomarker predictive. Therefore, the modification of microbiota composition can be used to improve the research of probiotic by creating new medications based on the patient's microbiota composition (if possible).

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