# Personalized therapy with TNF-inhibitors in Crohn's disease: Optimizing treatment outcomes by monitoring drug levels and anti-drug antibodies

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### THE 9 ORIGINAL PAPERS ARE

This doctoral dissertation is based on the following publications referred to in the text by Roman numerals:

- Steenholdt C, Enevold C, Ainsworth MA, Brynskov J, Thomsen OO, Bendtzen K. Genetic polymorphisms of tumour necrosis factor receptor superfamily 1b and fas ligand are associated with clinical efficacy and/or acute severe infusion reactions to infliximab in Crohn's disease. Aliment Pharmacol Ther. 2012;36:650-659.
- II. Steenholdt C, Brynskov J, Thomsen OØ, Munck LK, Fallingborg J, Christensen LA, Pedersen G, Kjeldsen J, Jacobsen BA, Oxholm AS, Kjellberg J, Bendtzen K, Ainsworth MA. Individualized therapy is more cost-effective than dose intensification in Crohn's disease patients who fail anti-TNF treatment: a randomized, controlled trial. Gut 2014;63:919-927.
- III. Steenholdt C, Brynskov J, Thomsen OØ, Munck LK, Fallingborg J, Christensen LA, Pedersen G, Kjeldsen J, Jacobsen BA, Oxholm AS, Kjellberg J, Bendtzen K, Ainsworth MA. Individualized Therapy Is a Long-Term Cost-Effective Method Compared to Dose Intensification in Crohn's Disease Patients Failing Infliximab. Dig Dis Sci. 2015;60:2762-2770.
- IV. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Munck LK, Christensen LA, Pedersen G, Kjeldsen J, Ainsworth MA. Changes in serum through levels of infliximab during treatment intensification but not in anti-infliximab antibody detection are associated with clinical outcomes after therapeutic failure in Crohn's disease. J Crohns Colitis 2015;9:238-245.
- V. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing in-

fliximab treatment failure in Crohn's disease: post-hoc analysis of a randomized controlled trial. Am J Gastroenterol 2014;109:1055-1064.

- VI. Frederiksen MT, Ainsworth MA, Brynskov J, Thomsen OØ, Bendtzen K, Steenholdt C. Antibodies against infliximab (IFX) are associated with de novo development of antibodies to adalimumab (ADL) and therapeutic failure in IFX-to-ADL switchers with inflammatory bowel disease. Inflamm Bowel Dis 2014;20:1714-1721.
- VII. Steenholdt C, Frederiksen MT, Bendtzen K, Ainsworth MA, Thomsen OØ, Brynskov J. Time course and clinical implications of development of antibodies against adalimumab in patients with inflammatory bowel disease. J Clin Gastroenterol. 2015 Jul 9. [Epub ahead of print]
- VIII. Steenholdt C, Molazahi A, Ainsworth MA, Brynskov J, Thomsen OØ, J Seidelin. Outcome after discontinuing infliximab in patients with inflammatory bowel disease in clinical remission: An observational Danish single center study. Scand J Gastroenterol. 2012;47:518-527.
- IX. Steenholdt C, Brynskov J, Thomsen OØ, Munck LK, Christensen LA, Pedersen G, Kjeldsen J, Ainsworth MA. Implications of infliximab treatment failure and influence of personalized treatment on patient-reported health-related quality of life and productivity outcomes in Crohn's disease. J Crohns Colitis. 2015;9:1032-1042.

# BACKGROUND

INFLAMMATORY BOWEL DISEASE

Crohn's disease and ulcerative colitis are idiopathic, chronic inflammatory bowel diseases (IBD) empirically defined by clinical, pathological, endoscopic, and radiological features, and characterized by a dysregulated immunoinflammatory response to mucosal antigens presumably within the commensal gastrointestinal bacterial flora in genetically susceptible individuals.[1-4] It is estimated that 4 per 1,000 Europeans and North Americans live with IBD.[5] The onset of IBD typically occurs in young adulthood.[6] Despite improved medical treatment options over recent years, the natural history of IBD appears largely unchanged and with a notable minority developing severe refractory disease activity or complications that ultimately require surgery.[6-12] IBD places a heavy burden on patients and society because it leads to life-long increased morbidity and disability including reduced health-related guality of life (HRQOL), and reduced capacity for work and impaired productivity.[1;6;13-16]

#### PHARMACOLOGICAL TREATMENT

Crohn's disease is traditionally managed by a 'step-up' strategy, whereby corticosteroids are initiated at initial diagnosis of active disease and at disease flares, conventional immunosuppressive agents such as thiopurines (azathioprine and mercaptopurine) or methotrexate (MTX) are added at corticosteroid dependency or recurrent flares, and therapy with biologic drugs in the form of tumor necrosis factor (TNF)- $\alpha$  inhibitors or recently approved leukocyte migration inhibitors are used if active disease persists despite these interventions or at an earlier stage in patients with severe fistulizing disease.[17-25] Similar principles generally apply to treatment of ulcerative colitis except that mesalazines are additionally used at acute exacerbations and for maintenance of remission, and MTX is not of proven value.[19;20;25-27] Although not routinely used, selected high risk patients may benefit from an accelerated 'step-up' approach, or from a 'top-down' regimen comprising early anti-TNF mono- or combination therapy.[28-36]

#### **TNF-INHIBITORS**

Introduction in the late 1990s of high molecular weight therapeutic monoclonal antibodies (Abs) which selectively inhibit the proinflammatory cytokine, TNF- $\alpha$  has revolutionized the treatment of IBD and other immune-mediated chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, and psoriasis.[37;38] By neutralizing both membrane-bound and soluble TNF- $\alpha$ , and supposedly by induction of apoptosis and cytolysis in selected immune cells, these agents down-regulate IBD associated inflammatory activity.[39-42] Accordingly, TNFinhibitors are efficacious for induction and maintenance of steroid-free clinical remission in patients with moderate to severe disease activity despite treatment with conventional agents, [21;43;44] facilitation of mucosal healing and closure of fistulas, [45-54] promotion of improved HRQOL, [55-60] and reduction of hospitalizations.[61-65] Approved TNF-inhibitors for IBD comprise the chimeric mouse-human immunoglobulin (Ig) G1-ĸ monoclonal Ab, infliximab (IFX); the fully human IgG1-κ monoclonal Abs, adalimumab (ADL) and golimumab (ulcerative colitis only); and a pegylated humanized monoclonal Ab Fab-fragment, certolizumab pegol (Crohn's disease in the USA only) (Figure 1).[66;67] All are administered parenterally at standard dosages, and with an initial high frequency induction scheme followed by regular maintenance therapy.[66;67]

Although clearly superior to placebo in state of the art randomized controlled trials (RCT), comparative effectiveness of TNFinhibitors has never been determined in head-to-head trials.[43;45;46;57;68-82] Indirect comparisons by network analyses suggest similar efficacy of IFX and ADL, but lower efficacy of certolizumab pegol as compared to these agents.[35;83-86] Initial choice of anti-TNF agent as well as preferred order of cycling through available agents in case of treatment failure thus depends on e.g. local guidelines and availability, costs, physician and patient preferences, and the order of regulatory approval.[35;83] IFX was the first TNF-inhibitor registered for treatment of IBD, and it is widely used as drug of choice having been on the market longest and with established efficacy and safety profiles. Hence, in the context of this dissertation it is acknowledged that much of the available data relate to IFX, with less for ADL, and that limited data is available for golimumab and certolizumab pegol.

#### FIGURE 1: ANTI-TNF-A BIOPHARMACEUTICALS



#### Figure 1 legend:

Infliximab is a chimeric monoclonal anti-TNF- $\alpha$  antibody (Ab) composed of a constant human immunoglobulin G (IgG)1-к lightchain spliced together with two identical variable fragment, antigen binding (Fab) regions encoded by genes in B-lymphocytes of a mouse immunized with human recombinant TNF-α. Infliximab consists of approximately 75% human and 25% murine amino acid sequences and is administered intravenously (human amino acid sequences are depicted in red, and murine sequences are shown in black/gray). Adalimumab and golimumab are subcutaneously administered human IgG1-к light-chain monoclonal Abs against TNF- $\alpha$ , and selected by phage display (adalimumab) or produced by transgenic-mouse technology (golimumab). Both drugs contain TNF-binding idiotopes that are not part of a normal human antibody repertoire (enlarged figure). Certolizumab pegol is a chimeric monoclonal Ab-fragment with an Ab-binding (Fab) region composed of a murine complementarity-determining variable region (CDR) specifically directed against human TNF- $\alpha$  grafted into a constant framework region (FR) of a human κ light-chain and IgG4 Fab. It is linked to polyethylene glycol (PEG) to prolong the halflife in the circulation (Table I). It is administered subcutaneously. Etanercept, which is not approved for treatment of IBD, is a human IgG1-fragment, crystallizable (Fc) region fused with the extracellular parts of two human TNF type 2, p75 receptors (TNF-R2). CL and CH: constant regions of IgG on light- and heavychains, respectively. VL and VH: variable regions of IgG on lightand heavy-chains, respectively.

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# CLINICAL RESPONSES TO TNF-INHIBITORS

Despite their proven efficacy and revolutionary impact on the therapeutic management of IBD, TNF-inhibitor treated patients are not immune to treatment failure.[43;87;88] Hence, about one

third of patients do not have a clinically relevant response to anti-TNF induction therapy and are classified as having primary treatment failure.[20;68-70;73;76;80;81;87-91] The basis for this definition is that pivotal placebo controlled maintenance trials with open label anti-TNF induction therapy observed maximal clinical response at week 10 for IFX, and at week 12 for ADL and certolizumab pegol.[31;45;57;70;73;77;89;92] In addition, up to half of patients with initial response lose effect during ongoing anti-TNF maintenance therapy and experience secondary treatment failure despite intensification of the treatment regimen. [43;88;92-96] Furthermore, a subset of patients only has partial effect of treatment with TNF-inhibitors despite continued treatment and fail to achieve complete remission. [45;57;70;72;73;75-77;80;82;89] Until recently, these issues received little attention, but this has changed as treatment goals in IBD have been extended from symptom control to persistent clinical, biochemical, and endoscopical remission with mucosal healing.[47;50;52;97;98]

EMPIRIC STRATEGIES FOR ANTI-TNF THERAPY OPTIMIZATION There is a need to secure long-lasting effectiveness of anti-TNF therapy to avoid treatment failure, and to rapidly regain treatment response in case of a declining effect.[87;99] These needs are important for several reasons. Firstly, there are limited alternative therapeutic options and until recently no other biologic agents against other targets than TNF were available. Secondly, prolonged periods with uncontrolled disease activity can cause structural complications with tissue damage and disease progression that is potentially irreversible.[6;12;100;101] Thirdly, as shown in study IX, anti-TNF treatment failure has immediate negative impact on patient-reported outcomes in the form of HRQOL and productivity, thus resulting in substantial patient impairment as well as indirect disease related costs for society, which can only be reversed once disease activity has been brought under control.[14;16;56;59] Furthermore, as the costs of anti-TNF therapies constitute one of the heaviest medical expenditures in many countries, cost-effective usage is required (II;III;IX) (Figure 2).[63;64;93;102-104] Along this line, the substantial costs of TNF-inhibitors along with concerns about potential treatmentrelated side effects, have led clinicians and national health-payers to consider cessation of anti-TNF treatment after certain treatment goals are achieved.[18;20;105-108]



### FIGURE 2: COSTS OF TNF-INHIBITORS IN DENMARK

Costs of anti-TNF therapies across all indications in the Danish secondary health care system. 1 Euro (EUR) equals approximately 7.5 Danish Kroner (DKK), and 1 US dollar (USD) equals approximately 7 DKK, as of medio 2015.

Source: Statens Serum Institut of Denmark (www.medstat.dk)

Rational usage of TNF-inhibitors along with strategies that optimize their efficacy and cost-effectiveness taking into account the different clinical response types is thus warranted. Guidelines generally suggest handling insufficient treatment effect or complete therapeutic failure of anti-TNF therapy by an empirically founded 'trial and error' strategy, where it is recommended to first intensify the treatment regimen with the existing anti-TNF agent, followed by switching sequentially to one or more other TNF-inhibitors, and then by switching out of class to biologic agents with other targets than TNF- $\alpha$ , if available. In parallel with this, it is suggested to optimize the use of concomitant treatment with conventional immunosuppressive agents, and to consider co-treatment with corticosteroids and surgery.[18-25] Selection of patients for treatment cessation during remission is usually done empirically by selecting those considered to have low risk of relapse based on disease and patient characteristics, and combined with physician and patient preferences. [18;20;26;49;109] However, it has been challenged if currently applied empiric strategies at treatment failure and at treatment cessation are optimal and cost-effective.[87;99;110;111]

#### PERSONALIZED ANTI-TNF THERAPY

A 'personalized' treatment approach for anti-TNF treatment optimization has evolved as a potential alternative to these empiric strategies. Here, it is speculated that rather than using standardized treatment regimens deduced from cohorts, patients may favorably be handled according to conditions in the individual patient through integration of selected relevant variables prior to treatment initiation and/or during ongoing therapy. These include e.g. clinical and disease related characteristics, genetic background, gene- and cytokine expression profiles, or measurements of drug concentrations or their metabolites in relevant tissue components.[112-116] Therapeutic drug monitoring (TDM) by measurements of anti-TNF drug levels and anti-drug Abs in peripheral blood for evaluation of pharmacokinetic (PK) and pharmacodynamic (PD) conditions in individual patients is considered an important component of personalized anti-TNF treatment in IBD.[87;90;99;116;117] The basis for this is that TNF-inhibitors consist of large glycoproteins that behave differently with regards to PK and PD as compared to conventional, chemically synthesized, small molecule drugs.[118-120] These characteristics of monoclonal Abs have been extensively reviewed elsewhere.[120-124] TDM is commonly used for other medications e.g. antibiotics, cyclosporine, tacrolimus, and digoxin.[125]

# THERAPEUTIC DRUG MONITORING *Pharmacokinetics*

The PK of anti-TNF agents is usually described by a twocompartment population PK model comprising peripheral blood as central compartment, and organs (i.e. the gut) in which the drug is distributed more slowly, as peripheral compartment.[126-129] The volume of distribution is small (~0.1 l/kg) and corresponds to distribution of TNF-inhibitors mainly in the extracellular fluid owing to their large molecular size and hydrophobic nature which precludes intracellular translocation.[123;130] Hence, anti-TNF drug levels in the circulation is considered a surrogate marker for the PK of TNF-inhibitors. Blood half-life (T½) is 15-20 days for fully human monoclonal anti-TNF Abs thus nearly resembling the half-life of naturally occurring IgG (~21 days); T½ is 10-20 days for humanized Abs, and 10-14 days for chimeric constructs.[126;130;131]

Figure 2 legend:

The impetus for TDM during anti-TNF therapy is the concentration-effect relationship. Multiple observational studies have reported an association between circulating anti-TNF drug levels and clinical outcomes, and with undetectable or low levels associated with insufficient effect or treatment failure and higher drug levels associated with favorable clinical outcomes (II;III;VI;VII).[31;81;132-145] However, anti-TNF drug levels in sera sampled at the end of the therapeutic cycle (i.e. trough levels) have been shown to vary considerably between patients on standardized weight-adjusted dose regimens, and even within the same patient across time. [113;120;146-148] These variations are determined by bioavailability and common PK parameters in the form of distribution, metabolism, and elimination from the blood. [118-120;130;149] Factors that influence the relationship between the administered anti-TNF dose and the achieved serum drug concentrations thus leading to intra- and inter-individual variations in drug exposure and potentially to variable clinical effectiveness are not completely understood. [130;150] However, emerging data summarized in Table I has identified a number of variables related to the patient, to the disease, and to the drug itself which can influence the PK of TNF-inhibitors.[120;127-130;133;148-162]

# TABLE I

| Variable   | Impact on pharmacokinetics<br>of TNF-inhibitors                                      |
|--|--|
| Gender   | Males have higher clearance<br>[127;164]   |
| Body mass index  | High body mass index increas-<br>es clearance  |
| Neonatal Fc receptor (FcRn) <sup>II</sup>                                  | Clearance may vary owing to<br>individual differences in activi-<br>ty and affinity  |
| Fcγ receptors <sup>III</sup>   | Clearance may vary owing to<br>individual differences in activi-<br>ty and affinity  |
| Albumin <sup>II;IV</sup>   | Low albumin levels increases clearance   |
| Inflammatory load <sup>IV</sup>  | High inflammatory load in-<br>creases clearance                                      |
| Immunogenicity   | Anti-drug Ab formation in-<br>creases clearance                                      |
| Combination therapy with conventional immunosup-<br>pressives <sup>V</sup> | Concomitant use of conven-<br>tional immunosuppressive<br>agents decreases clearance |
| Pegylation <sup>VI</sup>   | Decreases clearance  |
| Mode of administration <sup>VII</sup>                                      | Absorption after subcutaneous administration is variable                             |

# Table I legend:

<sup>1</sup> Proposed to be mediated by a higher inflammatory burden (see *IV*) and thus higher TNF-α levels in obese patients potentially by involvement of mesenteric adipose tissue as a source of proin-flammatory cytokine production.[130;159;160;266;268;421;422] Of note, patients with low weight are more likely to have low *IFX* 

trough levels because IFX clearance is not linearly related to weight, but IFX dosing is weight-based.

<sup>II</sup> Also denoted the Brambell receptor, and mediates homeostasis of IgG and albumin by a salvage mechanism primarily expressed by cells of the reticuloendothelial system (primarily vascular endothelial cells) that recycles these molecules back into the circulation thus prolonging their half-life (T½).[122;423;424] This system is saturable at high IgG concentrations resulting in an inverse relationship between concentration and half-life.[425] Hence, it is speculated that during severe inflammation, high circulating endogenous IgG may saturate FcRn binding sites and reduce retention of anti-TNF Ab thereby increasing their clearance (see IV).[130;149;365] It remains unknown if albumin's impact on the PK of anti-TNF agents is mediated via FcRn e.g. by an increased number of FcRn at high albumin levels, or rather is a surrogate marker of inflammatory activity (see <sup>IV</sup>).

<sup>III</sup> Fcy receptor I-III mediates binding with the Fc region of IgG followed by proteolytic catabolism after receptor-mediated endocytosis by mainly phagocytic cells of the reticuloendothelial system.[122;152;153;158;162;426;427] Proteolytic catabolism within the reticuloendothelial system is the primary route of clearance of monoclonal antibodies (Abs) including TNF-inhibitors (certolizumab pegol is an exception because it lacks the Fc region).[130;149]

<sup>IV</sup> The presence of systemic inflammation (increased CRP levels, lowered albumin levels) increase overall Ig catabolism in the reticuloendothelial system and thereby in itself increase clearance of anti-TNF agents.[130;133;151;164;195;239;315;365;428] Furthermore, high levels of TNF- $\alpha$  in the severely inflamed gut warrant increased anti-TNF dosage due to the presence of high antigen levels (i.e. TNF- $\alpha$ ). In addition, high intestinal TNF- $\alpha$  levels increase anti-TNF drug clearance by internalization and proteolytic degradation in lysosomes by binding of the anti-TNF Ab to membrane-associated TNF- $\alpha$  or by degradation of immune complexes (TNF- $\alpha$ /anti-TNF agent) by the reticuloendothelial system.[94;120;123;128;154;157;158;203;429] Collective, these processes are denoted the 'antigen-dependent clearance pathway' or the 'antigen sink'. In addition, increased intestinal permeability during active intestinal inflammation leading to fecal loss of anti-TNF Abs (and potentially also albumin) also contribute to anti-TNF drug clearance being depended of the inflammatory load.[161;399;430] Along this line, observations indicate that patients with ulcerative colitis often have higher clearance of TNFinhibitors than patients with Crohn's disease at periods with severe inflammatory activity. The higher clearance is speculated to arise, at least in part, from a higher overall inflammatory burden for the reasons mentioned above combined with involvement of a larger intestinal inflammation area in these patients.[136;149;365;386]

<sup>v</sup> Mediated likely by reduced anti-drug Ab formation and/or reduced inflammatory load and/or down-regulation of reticuloendothelial-mediated drug clearance by e.g. altering receptors for Ig such as Fcy receptors (see <sup>III</sup>).[130;158]

<sup>VI</sup> Mediated by increased molecular mass above glomerular filtration limit, protection from proteolytic breakdown, and protection from immunological recognition, e.g. certolizumab pegol.[431] <sup>VII</sup> Subcutaneous absorption of monoclonal Abs occurs primarily via lymphatic drainage and into the blood.[432] Approximately 50-100% of the administered dose is absorbed with the remaining dose being lost through pre-systemic catabolism.[120] In addition, the subcutaneous route is often more immunogenic than intravenous administration.[118]

# Anti-drug antibodies

In some patients, one dominant factor shown to affect the PK of TNF-inhibitors and result in increased drug clearance is immunogenicity with formation of anti-drug Abs (Table I) (II;III;IV;V;VI;VII).[88;118;120;127;129;146;160;163;164] Antibody formation against TNF-inhibitors are caused by recognition of the drug as a foreign substance by the recipient's immune system usually initiated by T cell recognition of non-self peptides displayed on antigen-presenting cells and followed by B cell activation with production of mainly IgG targeting the anti-TNF agent.[119;165-168] Thus, this aspect of TNF-inhibitor therapies resembles common vaccination procedures where repeated administrations of non-self proteins are used to elicit an immune response.[169]

Anti-drug Abs have been reported in approximately one third of IBD patients on maintenance therapy with IFX, and with somewhat lower frequencies for ADL and golimumab, and even lower frequency for certolizumab pegol

(II;III;IV;V;VI;VII).[76;77;135;139;139;142;144;170-173] However, the incidence is difficult to estimate due to the use of different types of anti-drug Ab assays, diverse study populations, and variable timing of sampling during the course of treatment (IV;V;VI;VII). The levels of anti-drug Abs in the circulation are decreased by use of maintenance therapy as opposed to episodic therapy, by avoidance of prolonged periods with sub-therapeutic dosing, and, notably, by concomitant immunosuppressive treatments with thiopurines or MTX; although the latter was not observed in study VI and VII likely owing to small sample sizes.[20;49;88;117;130;139;170;172;174-176] It remains to be unequivocally established if pre-infusion of high dose corticosteroids prior to IFX infusions reduce risk of anti-IFX Ab formation as suggested in a clinical trial.[177-179] Anti-drug Abs can sometimes be detected several years after treatment cessation.[180-182]

Anti-drug Ab formation can arise from recognition by the immune system of non-human molecular structures as is usually the case for IFX, where the main immunogenic component is the murine part of the Fab fragment (Figure 3).[165;167] Anti-TNF biopharmaceuticals consisting of human sequences only, can elicit antidrug Ab formation from recognition of e.g. non-self allotypes, neoepitopes generated by drug aggregation, non-human glycosylation, or from non-self Ig idiotypes within the TNF-binding region as is predominately occurring for ADL (Figure 3).[114;183-186] As illustrated in Figure 3, depending on the exact binding site and binding kinetics, anti-drug Abs can be non-neutralizing or, alternatively, interfere directly with the binding of the anti-TNF molecule to its target TNF- $\alpha$  molecule thereby neutralizing the biologic activity of the drug. Irrespectively, both neutralizing and non-neutralizing anti-drug Abs have the capacity to increase systemic drug clearance by immune complex formation that is rapidly degraded and removed from the circulation, for example by filtering in the spleen (Table I).[88;120;123;127-129;160;187-190] Furthermore, in case of subcutaneous administration, antidrug Abs are likely to augment systemic variations in drug bioavailability by local formation of immune complexes and impaired absorption, irrespectively of the binding site(s) on the anti-TNF molecule.[114]

Anti-drug Abs have generally been associated with undetectable or low drug levels, and with decreased clinical efficacy or manifest treatment failure (II;III;IV;V;VI;VII).[117;139;144-146;171] In addition, anti-IFX Abs markedly increase the risk of acute infusion reactions during IFX therapy.[170;191-193]

# FIGURE 3: PUTATIVE IMMUNOGENIC SITES ON ANTI-TNF-A BIO-PHARMACEUTICALS



# Figure 3 legend:

Antibodies against anti-TNF- $\alpha$  biopharmaceuticals (ADA) are shown as yellow. Anti-TNF- $\alpha$  constructs, drugs, and drug fragments with 'human' aminoacid sequences, are depicted in red. Mouse sequences are shown in black/gray. CDR: Complementarity-determining variable region of antibody; CH1, CH3 and CL: Constant regions of IgG on light and heavy chains, respectively; Fab: antigen-binding region of antibody; FC: crystallizable region of antibody; FR: Framework region of antibody; TNF: tumornecrosis factor; TNF-R2: TNF type 2, p75 receptor. VH, VL: variable regions of IgG on heavy and light chains, respectively.

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# Pharmacodynamics

Although therapeutic anti-TNF Abs have been commercially available for several decades, little is known about their PK-PD relationship.[130] However, it is increasingly recognized that not only the PK of TNF-inhibitors differs between individual patients and thereby cause variable clinical outcomes of anti-TNF therapy, but that the same holds true also for the PD of TNFinhibitors.[87;88;90;115;120;130;149] This is substantiated by observations that effectiveness of anti-TNF therapy at similar serum drug levels varies considerably between individuals (II;III;IV;V;VI;VII).[135;136;138;139;143;144] Furthermore, a seemingly large proportion of patients with primary or secondary anti-TNF treatment failure have very high circulating drug trough levels, supporting that ongoing inflammatory activity is independent of TNF- $\alpha$  and is thus likely caused by a PD mechanism (II;III;IV;V;VI;VII).[88;91;134-136;138;139;143;144] Accumulating data on the expression of cytokines including TNF- $\alpha$ , as well as gene expression studies, support that PD mechanisms for inadequate effectiveness of anti-TNF therapy can be caused by predominantly or exclusively non-TNF-driven inflammatory disease pathways – either primarily or as a result of redundancy with a dynamic shift during ongoing anti-TNF therapy.[154;194-204]

# AIM

The overall aim of this dissertation was to examine if anti-TNF therapy can be tailored on an individual IBD patient basis by considering prognostic factors prior to initiation of therapy, during ongoing anti-TNF treatment, and in the context of treatment cessation for improved treatment outcomes and improved cost-effectiveness.

For this purpose, it was primarily intended to investigate the role of TDM by measurements of anti-TNF drug and anti-drug Abs. In particular, the aim was to test the hypothesis that TDM-based strategies could aid prospective therapeutic guidance in case of treatment failure during ongoing anti-TNF maintenance therapy.

Also, the study sought to characterize and compare different assays for detection and quantification of anti-TNF drug and antidrug Abs, and to identify potential pitfalls related to technical and temporal aspects of such measurements when using TDM-based strategies to optimize anti-TNF therapy.

Furthermore, the aim of the study was to quantify the consequences of anti-TNF treatment failure for society in terms of treatment related costs and for patients in terms of HRQOL. Lastly, the study explored if assessment of patient, disease, or treatment related characteristics support a personalized anti-TNF treatment approach and taking into account different clinical anti-TNF response types.

The study focused on patients with Crohn's disease, and was carried out in a setting where IFX was used as first choice of TNF-inhibitor followed by ADL as second line agent.

# SEARCH STRATEGY

In order to review the current literature in light of the findings of studies I-IX that form the basis for this doctoral dissertation, a systematic review of English language, full length articles, indexed in PubMed as of July, 2015 was carried out. The following search terms alone or in combination were used and without restriction on the year of publication: "IBD", "Crohn's disease", "ulcerative colitis", "infliximab", "adalimumab", "golimumab", "certolizumab pegol", "anti-TNF", "vedolizumab", "biologics", "therapeutic drug monitoring", "pharmacokinetics", "pharmacodynamics", "antibody", "immunogenicity", "primary non-response", "secondary non-response"," loss of response", "partial response", "cessation", "discontinuation", "prediction of response". The reference lists of all relevant articles were also examined. Studies concerning the use of TNF-inhibitors in children with IBD, and in other diseases than IBD, were included only if relevant information not available in studies of adult IBD was reported.

# MEASURING ANTI-TNF DRUG AND ANTI-DRUG ABS TECHNOLOGIES

A fundamental aspect of applying TDM-based strategies for anti-TNF treatment optimization is the ability of assays to accurately and reliably measure levels of functionally active anti-TNF drug with TNF-neutralizing capacity; and to detect functionally active anti-drug Abs with drug-neutralizing capacity and/or capacity to increase anti-TNF drug clearance.[114;205-209] The importance of these issues are reflected in the regulatory guidelines for approval of biological drugs.[210;211] Several techniques are being used for this purpose, and there is no defined gold standard assay.[88] Consequently, it is not surprising that the choice of assay can be influence by factors not directly related to analytical quality and clinical correlation, e.g. availability, price, local expertise and facilities, complexity etc.

Enzyme-linked immunosorbent assays (ELISA) comprise the most widely available and most commonly used type of assay.[117;139;149] These rather inexpensive solid-phase binding assays are relatively easy to establish in local research laboratories and guite simple to use. Hence, a large number of different subtypes of in-house and commercially available ELISA tests have been used in clinical studies.[69;115;212] The measurement of anti-TNF drug levels is usually done by capture ELISA, whereby a detection Ab is used to measure the level of drug in serum captured by plate-bound TNF (Figure 4).[70;132;133;175;213;214] The same capture ELISA principle cannot be used to measure antidrug Abs, as both the antigen (the anti-TNF agent) and the analyte of interest (the anti-drug Abs) consist of IgG, and so a labelled anti-human IgG detection Ab will bind both the drug and the antidrug Abs.[90] Various modified ELISAs have been constructed to circumvent this problem, e.g. bridging ELISA where the drug serves as both the capture antigen and in labelled form as the detection Ab (Figure 5);[72;115;132;133;140;175;175;177;213-215] or a modified capture ELISA employing anti-human  $\lambda$  light chain conjugated Ab in the detection phase utilizing that TNFinhibitors are composed of  $\kappa$  light chains (Figure 6).[167;216-218]

In recognition of potential limitations of ELISA, alternative analytical techniques for measuring anti-TNF drug and anti-drug Abs have been introduced in recent years. These assays are usually technically more complex and often require specialized laboratories, thus making them more expensive and more laborious with longer latency for test results. Notable examples comprise two types of fluid-phase binding assays in the form of radioimmunoassay (RIA) and homogeneous mobility shift assay (HMSA).[111;115;117;139;219] In brief, RIA measures drug levels as the capacity of patient serum to bind radiolabeled TNF, and detects anti-drug Abs by their binding to radiolabeled anti-TNF drug followed by precipitation and quantification using antihuman  $\lambda$  light chain Abs (Figure 7).[134;138;165;193] HMSA is a high pressure liquid chromatography (HPLC)-based mobility shift assay which incorporates an acid dissociation step that separates drug/anti-drug Ab-complexes and based on molecular size then quantifies drug by its binding to fluorescence-labelled TNF, and anti-drug Abs by binding to fluorescence-labelled anti-TNF drug (Figure 8).[141;172;220] Finally, a principally different type of analytical technique in the form of a functional cell-based reporter gene assay (RGA) measures TNF-α-mediated effects on a TNFreceptor-positive human cell line enabling detection of both TNF- $\alpha$  neutralization by the drug, and neutralization of this effect by anti-drug Abs (Figure 9).[221]

FIGURE 4: ELISA FOR QUANTIFICATION OF ANTI-TNF ANTIBODY CONSTRUCTS



# Figure 4 legend:

Examples of capture enzyme-linked immunosorbent assay (ELISA) for quantification of anti-TNF drug levels (infliximab and etanercept) using wells coated with human recombinant TNF- $\alpha$  (left and middle panel) or a 'capture' monoclonal antibody (right panel), and a biotinylated or enzyme-labeled anti-human IgG detection antibody.

Reproduced with permission from Bendtzen K.

# FIGURE 5: BRIDGING ELISA FOR DETECTION OF ANTI-DRUG AN-TIBODIES



# Figure 5 legend:

Bridging enzyme-linked immunosorbent assay (ELISA) for detection of anti-drug antibodies (ADA) depends on the bivalency of IqG ADA (and multivalence of IqA and IqM ADA) and therefore the ability of these immunoglobulins to 'bridge' drug molecules preabsorbed to a plastic well with an added enzyme-labelled anti-TNF drug molecule in the detection phase (panel 1). False positive ADA testings can arise from cross-binding of anti-TNF drug Fcfragments by sera containing rheumatoid factor, anti-allotypic Abs, or activated complement (C1qr2s2) due to inflammatory activity (panel 2). False negative ADA testings can be caused by drug sensitivity of the assay implicating that ADA bound to anti-TNF drug in patient sera do not bind to anti-TNF drug preabsorbed to the solid phase and thus cannot generate cross binding to the labelled anti-TNF drug molecule in the detection phase (panel 3). False negative ADA testings can also be due to failure to detect functionally monovalent IgG4 anti-drug Abs (panel 4).

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# FIGURE 6: CAPTURE ELISA FOR DETECTION OF ANTI-DRUG ANTI-BODIES



Caveats



### Figure 6 legend:

Capture enzyme-linked immunosorbent assay (ELISA) for detection of anti-drug antibodies (ADA). Upper panel:  $\lambda$  light chain ADA, bound to the anti-TNF agent captured on TNF- $\alpha$ -coated plastic wells, are detected by enzyme-labeled anti-human  $\lambda$  light chain antibody (Ab) exploiting that anti-TNF drugs are comprised of lgG1- $\kappa$  light chains. Lower panel: False negative ADA testings may arise from failure to detect anti-idiotypic ADA, or from drug sensitivity of the assay rendering ADA bound to anti-TNF drug in patient sera undetectable.

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# FIGURE 7: RIA FOR DETECTION OF ANTI-DRUG ANTIBODIES



#### Figure 7 legend:

Fluid-phase radioimmunoassay (RIA) for detection of anti-drug Abs (ADA). The example shows RIA for ADA against infliximab (IFX). Patient serum containing ADA is first incubated with radiolabeled IFX. Then, free and immunoglobulin-bound tracers are separated by spinning down only the radiolabeled drug binding to  $\lambda$  light-chain ADA in complex with anti- $\lambda$  light-chain Ab.

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# FIGURE 8: HMSA FOR DETECTION OF ANTI-DRUG ANTIBODIES



#### Figure 8 legend:

Homogeneous mobility-shift assay (HMSA) for drug-binding antidrug antibodies (ADA) depends on association of fluorescencelabeled drug added to serum and subsequent chromatographic separation of fluorescence-labelled drug in free form and in complex with ADA (left panel). Note that functionally inactive ADA, bound to drug in vivo, may be split during assay and reassociated with tagged drug before or during chromatography (right panel), thus reporting similar data as visualized in the left panel.

Reproduced with permission from Bendtzen K from: Bendtzen K. Immunogenicity of Anti-TNF-alpha Biotherapies: II. Clinical Relevance of Methods Used for Anti-Drug Antibody Detection. Front Immunol 2015;6:109 (doi: 10.3389/fimmu.2015.00109).[235]

# FIGURE 9: RGA FOR DETECTION OF ANTI-TNF DRUG AND ANTI-DRUG ANTIBODIES



### Figure 9 legend:

The cell-based reporter gene assay (RGA) measures functional levels of anti-TNF drug, and functional levels of all classes of drugneutralizing anti-drug antibodies (ADA). When human recombinant TNF is added to the target cells, the cytokine initiates intracellular signaling through the surface TNF-receptor, type1 (TNF-R1), thus activating the cytoplasmic nuclear factor (NF)-KB (step 1: left). The active components of this transcription factor are then transported into the nucleus where they bind to NF-κB response elements (NF-KB-REs) in the genome. This activates more than a hundred genes, including an inserted reporter-gene construct encoding the enzyme Firefly luciferase. After cell lysis and addition of substrate, luciferase-catalyzed light emission can be quantified (Steps 2 and 3). When TNF is preincubated with patient serum containing an anti-TNF drug and then added to the cells (step 1: middle), the drug, if functional, neutralizes the effect of TNF, and no intracellular signal is initiated. When TNF is preincubated with patient serum containing drug-neutralizing ADA and then added to the cells (step 1: right), the drug no longer interferes with TNFmediated signaling, resulting in a luminescence signal.

Reproduced with permission from Bendtzen K from: Bendtzen K. Immunogenicity of Anti-TNF-alpha Biotherapies: II. Clinical Relevance of Methods Used for Anti-Drug Antibody Detection. Front Immunol 2015;6:109 (doi: 10.3389/fimmu.2015.00109).[235]

# QUANTIFICATION OF ANTI-TNF DRUG LEVELS

Comparisons of assays for anti-TNF drug detection In a series of studies, representative formats of ELISA, RIA, HMSA, and RGA for quantification of IFX were compared (IV;V).[222] Taken as a whole, basic analytical parameters such as limit of detection (LOD), imprecision, and inaccuracy were largely comparable between assays. Furthermore, these assays showed highly significant linear correlations (Person's r>0.9), and to a large degree agreed on ranking of IFX levels in samples obtained from Crohn's disease patients with symptomatic IFX treatment failure (intraclass correlation coefficient >0.7). Even though RIA and HMSA showed higher sensitivity for detection of IFX than ELISA and RGA in sera obtained from 66 Crohn's disease patients with IFX treatment failure, IFX was detected by all assays in 80% of IFX positive samples by any of the assays used. Notably, all pair of assays systematically and highly significantly disagreed on sample IFX concentrations with a mean difference ranging from 0.6 µg/mL in ELISA and HMSA up to 3.4 µg/mL in RGA and ELISA. Along this line, even different subtypes of capture ELISAs have been shown to differ in their ability to detect and quantify IFX.[223]

# Methodological biases related to anti-TNF drug detection

These observations reveal a relatively high degree of congruence of anti-TNF drug detection between different types of assays. This concords with the consistent associations between anti-TNF drug levels and clinical outcomes independently observed in numerous studies and irrespectively of applied assay (II;III;IV;V;VI;VII).[117; 132-134;136;138;140-142;144;172;213;221] The underlying reasons for minor discrepancies in anti-TNF drug detection as well as systematic disagreement on exact sample concentrations may relate to differences in sensitivity observed between the assays (IV;V;VII).[222] However, several other factors related to the principally different designs and thus differences in technical properties may also contribute.[114;115;165;219;224] For example, ELISAs are generally prone to matrix effects and interference by factors in the serum such as rheumatoid factors and complement components.[184;206-209] These solid-phase binding assays are also more artificial than the fluid-phase assays because of the potential to both mask epitopes which are normally displayed in vivo, and to introduce new epitopes not present in vivo.[224] Fluid-phase assays (RIA and HMSA) are believed to resemble conditions in vivo better, and these assays measure the TNF-binding capacity of the drug which better relates to the functional TNF-neutralizing effect as opposed to solid-phase assays that measures a protein which may or may not be functional.[166;184] As HMSA involves an acid dissociation step that separates complexes of drug and anti-drug Abs, only this assay measures circulating non-functional anti-TNF drug completely neutralized by anti-drug Abs (Figure 8). On the other hand, drug detection by RGA is an assessment of the biologically active anti-TNF activity available in serum that interferes with cellular receptors in vivo (Figure 9).[115]

# QUANTIFICATION OF ANTI-DRUG ABS Comparisons of assays for anti-drug Ab detection

Detection of antibodies against TNF-inhibitors is generally impeded by the fact that the drug is an Ig in itself, and by the complexity of measuring Abs against Abs in binding assays.[115;166;205-209] In addition to the lack of a gold standard assay, there is no standardized reporting of anti-drug Ab detections and with most studies providing results as a binary variable (positive or negative) according to the LOD of the assay, others use arbitrary concentrations (V).[88;225] In a study comparing basic properties of common assays for anti-IFX Abs, RIA was substantially more sensitive and thus able to detect anti-IFX Abs in lower titers as compared to bridging ELISA and RGA.[222] Furthermore, in line with the findings for quantification of anti-TNF drug levels, concentrations of anti-IFX Abs determined as titers, and using a common readout point in all assays to facilitate inter-assay comparisons, showed significant disagreement between assays and correlations were relatively low.[222] Different formats of bridging ELISAs have also been shown to disagree on detection and guantification of anti-IFX Abs.[223] Coherently, anti-IFX Ab detection in samples obtained from 66 Crohn's disease patients with IFX treatment failure was highly variable ranging from 9% of patients in ELISA and 11% in RGA, to up to 27% in RIA and 33% in HMSA (IV;V). Of note, all anti-IFX Ab positive patients by ELISA and RGA were also all found positive in RIA and HMSA, suggesting a higher sensitivity of the two latter assays and/or that these assays reported functionally inactive anti-IFX Abs in addition to neutralizing anti-IFX Abs (IV;V).

# Methodological biases related to anti-drug Ab detection

Formation of anti-drug Abs has been associated with diminished or eliminated drug detection, and loss of clinical response or manifest treatment failure in most studies, albeit not in all (II;III;IV;V;VI;VII).[136;139;144;170;171;226] This has led to an ongoing debate about the effect of anti-drug Abs on clinical outcomes.[94;139;158;227] Based on the head-to-head comparisons of anti-IFX Ab detection by commonly used assays, it is likely that reported variations of the clinical importance of anti-drug Abs at least to some extent stem from analytical incongruence between the assays utilized, thereby biasing reported (lack of) associations (II;III;IV;V;VII).[222] Potential methodological biases related to anti-drug Ab detection have been addressed in previous reviews.[114;115;219;224] Hence, a number of aspects need consideration as outlined below and explored in studies IV, V, and VII.

ELISA does not detect anti-drug Abs in the presence of drug.[114;115;184;228] This can give rise to false negative results, or false low levels, as illustrated in Figures 5 and 6. Therefore, serum samples are best obtained as 'trough levels' with sampling immediately prior to the next administration as drug levels are lowest here.[88;139] Irrespectively, anti-drug Abs are reported as inconclusive by ELISA in up to half of patients because of detectable drug and negative anti-drug Ab testing.[89;133;174;175] As shown in study V, false negative results may also originate from bridging ELISA's inability to detect functionally monovalent IgG4 anti-drug Abs which, together with IgG1, are the prominent isotypes of Ig after prolonged immunizations. Notably, these were present in 63% of anti-IFX Ab positive Crohn's disease patient sera (V) (Figure 5).[165;166;222;228-232]

Most currently used capture ELISA, and RIA, employ anti-human  $\lambda$  chain conjugated Ab in the detection phase and thus do not detect  $\kappa$  light chain anti-drug Abs.[138;146;165;216] As anti-drug IgG Abs express  $\kappa$ - and  $\lambda$  light chains at a constant ratio similar to that of natural Abs, and because binding avidities are largely independent of the light chain isotype, false negative results due to lack of  $\kappa$  light chain anti-drug Ab detection are considered unlike-ly.[146;165] ELISAs and other solid-phase assays are also known to report false negative results due to e.g. neoepitope formation or cross-binding of IgG Fc by activated complement or by non-specific binding of low-affinity Abs including heterophilic Abs and

rheumatoid factors (Figures 5 and 6).[115;146;166;205;228;233; 234] Finally, ELISA does not assess if detected anti-drug Abs are functional and interfere with anti-TNF drug activity in vivo (IV;V;VII).[114;115;184;205;224]

High sensitivity binding assays in the form of RIA and HMSA take place in fluid phase thereby resembling in vivo conditions better than ELISA, and are not to the same degree influenced by the potential artifacts encountered in solid-phase assays (Figures 7 and 8).[165;220] Both types of assay measure all isotypes of antidrug Abs (V).[165;219;220] Anti-drug Abs detected by RIA and HMSA are functional in the sense that they are capable of binding to labelled anti-TNF drug during the assay procedure. However, this may not necessarily extrapolate to functionality in vivo. For example, the artificial split of anti-drug Abs in complex with drug in HMSA may result in detection of non-functional anti-drug Abs which are part of a 'neutral' drug/anti-drug Ab complex possibly without clinical consequences (Figure 8).[235] Data from study IV, V, and VII suggest that this may indeed occur in both HMSA and RIA. However, concomitant detection of anti-TNF drug and antidrug Abs can actually be a harbinger of loss of treatment response, and these apparent non-functional anti-drug Abs may be of clinical relevance owing e.g. to non-neutralizing binding to the anti-TNF drug followed by increased drug clearance and, hence, diminished drug exposure.[118;208;209;216;236] Thence, the clinical relevance of low concentration anti-drug Abs not detectable in drug sensitive assays remains to be explored in more detail. HMSA and other assays incorporating immune complex dissociation may suffer from incomplete dissociation of the complexes or reassociation before completion of the assay; and the process of pH-shifting can also introduce artefacts (Figure 8).[235;237] Even though RIA measures anti-drug Abs in the presence of limited amounts of anti-TNF drug, study VII shows that interference of drug can cause false negative results in RIA, albeit less so than in ELISA.[165;221]

As mentioned, currently applied binding assays for anti-drug Abs in the form of ELISA, RIA, and HMSA do not discriminate between neutralizing and non-neutralizing anti-drug Abs (IV;V;VII). This is important because binding assays do not assess whether or not an observed binding ex vivo between drug and anti-drug Abs has a clinically relevant effect in vivo. However, the vast majority of patients with detectable anti-drug Abs by RIA in studies II-VII, or HMSA in studies II, IV, and V, had low or undetectable drug levels indicating functionality of detected anti-drug Abs by these assays as defined by their ability to increase drug clearance and/or neutralization. In contrast, RGA detects TNF-α activity, not drug or anti-drug Abs sui generis as do the binding assays, and its test outcome is a functional assessment of biologically active IFX and anti-IFX Abs that interfere with cellular receptors thus resembling conditions in vivo (II;III;IV;V;VII).[115;221] However, RGA measures only neutralizing anti-drug Abs, and even though neutralizing anti-drug Abs are assumed to be highly clinically relevant, non-neutralizing anti-drug Abs may also be functionally active and have clinical implications due to increased clearance of the anti-TNF drug. [88;120;123;127-129;188-190]

# TEMPORAL ASPECTS RELATED TO INTERPRETATION OF ANTI-DRUG AB TEST RESULTS

Bearing in mind potential methodological and technical biases when evaluating the clinical relevance of anti-drug Ab test results, accumulating data including observations presented in studies IV and VII also stress the importance of recognizing biases related to systemic appearance and evolution of anti-drug Abs. Hence, as anti-drug Ab generation is a dynamic process developing and changing over time, a number of pitfalls relate to timing of sampling during the course of treatment. These may generate false positive or false negative anti-drug Ab test results from a clinical perspective as summarized below.

# Chronology between anti-drug Ab detection and treatment failure

In about half the patients, detection of anti-drug Abs precede the onset of symptoms of treatment failure by months, likely owing to a time lag between appearance of anti-drug Abs, low anti-TNF drug levels and insufficient inhibition of TNF- $\alpha$  mediated intestinal inflammation, and then re-emergence of considerable tissue inflammation above a threshold at which symptoms of clinical relapse becomes apparent.[180;238] Furthermore, while simultaneous timing of anti-drug Ab detection and manifestation of treatment failure occurs in approximately one third of patients, anti-drug Abs are detected several months after symptomatic treatment failure in about one fifth of patients. This may be owing to drug sensitivity of the assay utilized, that drug/anti-drug Ab-complexes have been cleared from the circulation at the time of initial measurements due to initial low titers of anti-drug Abs, or that persistent sub-therapeutic dosing in itself has provoked an immune response that leads to formation of anti-drug Abs (IV;V;VII).[238] Several studies have reported that low serum trough level of anti-TNF drugs is often a precursor for later development of anti-drug Abs.[94;136;136;175;215;239-241]

# Systemic appearance of anti-drug Abs

It has been reported, that anti-drug Abs generally become detectable in the circulation among scheduled-treated patients within the first year of anti-TNF therapy (VII).[132;174;238] Hence, the risk of drug immunogenicity is very low if one year of anti-TNF therapy has elapsed without formation of anti-drug Abs and this may at least partly explain the demonstrated lack of superiority of combination between TNF-inhibitors and conventional immunosuppressives beyond 6-12 months (Table I).[242-244] In addition, successful retreatment with IFX after discontinuation of IFX therapy in patients in long-term sustained clinical remission as observed in study VIII, may also be explained by the temporal appearance of anti-drug Abs because successful completion of a longer period on IFX probably selects a subgroup with low risk of anti-drug Ab formation (VI;VII).[90;245] Along this line, variations in reported frequencies of anti-drug Ab formation may relate not only to technical differences between assays but also to diverse time points of sampling during the course of treatment (VII).[135;143;144;170;171;214]

# Transiency of anti-drug Abs

In an estimated one fourth of patients, anti-IFX Abs may disappear at later reassessment during continued IFX therapy.[180;226;238;240] Study VII extends this phenomenon to include also anti-ADL Abs.[246] Interestingly, these transient anti-drug Abs appear to have little clinical relevance and do not associate with treatment failure as opposed to persistent anti-drug Abs (IV;VII).[180;238;240] Observations presented in study IV and VII show that transient anti-drug Abs in some patients present as neutralizing anti-drug Abs, and in other patients as biologically inactive Abs because they are circulating as immune complexes bound to the anti-TNF agent in vivo. Even though data are conflicting, it seems that transient anti-drug Abs are initially detected

at lower titers than persistent anti-drug Abs, and that transient anti-drug Abs can present after years of anti-TNF therapy as opposed to persistent anti-drug Abs which generally become detectable in the circulation within the first year of therapy (IV;VII).[180;238;240]

It has been suggested that anti-drug Ab transiency is a result of drug-induced immunological tolerance for example by activation of regulatory T cells. [180;240;247-250] This hypothesis is in accordance with observations in study IV that anti-IFX Abs detected at the time of IFX treatment failure were undetectable at reassessment after 12 weeks of intensified IFX regimen, both when assessed by a functional assay (RGA) and by a drug tolerant binding assay which separates complexes of drug/anti-drug Abs (HMSA). In support hereof, it has been reported that addition of conventional immunosuppressive agents at IFX treatment failure in the presence of neutralizing anti-IFX Abs was followed by the elimination of anti-drug Ab detection, restored detection of drug, and regained clinical response over time.[251] However, it should be noted that the same result could be explained by the antiinflammatory activity of the immunosuppressive agent, as this might reduce the TNF- $\alpha$  load thus lowering the need for anti-TNF drug, elevating the circulating drug levels with increased elimination of anti-drug Abs.[252]

However, based on available data, the hypothesis of a capacity to induce immunological tolerance to TNF-inhibitors once anti-drug Abs have developed cannot be proved at present, as this would require direct testing of antibody production by anti-TNFchallenged immune cells from patients with assumed tolerance. Of note, several alternative explanations for the observed antidrug Ab transiency exist. For example, drug/anti-drug Ab-immune complexes may have been completely cleared from the circulation at the time of trough sampling, e.g. due to increased anti-TNF drug load during an intensified treatment regimen, thus being undetectable but with anti-drug Abs produced continuously at subsequent anti-TNF drug administrations (IV;VII). This, along with a false negative anti-drug Ab test result at time of reassessment due to e.g. interference with drug present in the sample as was shown to sometimes occur for anti-ADL Abs in study VII, will give rise to misinterpretations, as anti-drug Abs would still be produced and thus still be present in the circulation and potentially lead to efficacy and safety problems if anti-TNF therapy is continued. Finally, as shown in study VII, an initial false positive anti-drug Ab testing will also be misinterpreted as transiency at subsequent negative anti-drug Ab testings.

Taken together, the fact that our original demonstration of antidrug Ab transiency has been confirmed in several independent cohorts in IBD and other diseases, in serial samples and by use different assays, and during therapy with other biologic agents than TNF-inhibitors, indicate that anti-drug Abs in some situations are available in the circulation only transiently and without clinical implications (IV;VII).[180;226;238;240;246;253-255] However, even if anti-drug Ab transiency is a consequence of methodological or temporal biases, this phenomenon still complicates interpretation of anti-drug Ab test results in the clinical setting.

# PITFALLS WHEN APPLYING TDM-BASED STRATEGIES FOR ANTI-TNF THERAPY OPTIMIZATION

Potential TDM-related biases and corresponding implications Data presented in studies II-VII have contributed to an understanding that serum trough measurements of anti-TNF drug levels comprise a clinically relevant and relatively robust surrogate marker of the PK of anti-TNF agents. Further, that this is largely unaffected by the analytical technique utilized although minor discrepancies does exist. On the other hand, that assessment of anti-drug Abs to identify PK problems related to drug immunogenicity is more complex, and thus more challenging to use in clinical practice. This is presumably because of methodological incongruence and risk of biases related to detection of anti-drug Abs by available assays without a gold standard. Furthermore, there is a risk of biases related to the temporal characteristics of anti-drug Abs in the form of e.g. their systemic appearance and potential disappearance during the course of treatment, and lack of chronology between presentation of symptoms and presentation of circulating anti-drug Abs. Collectively, these issues along with lack of uniform clinical outcome definitions, diverse patient populations, and diverse trial designs are likely to have contributed to reported inconsistencies in the literature regarding association between anti-TNF drug and anti-drug Abs and clinical outcomes.

It is not yet possible to define an optimal analytical technique for detection and quantification of anti-TNF drug and anti-drug Abs to be used for prospective clinical guidance of therapeutic interventions (II;III;IV;V;VI;VII). However, the capacity of an assay to estimate the functional effects of anti-TNF drug and anti-drug Abs in vivo is considered to be essential in this respect. [210;211] Importantly, test results – both when classified as binary variables (positive vs. negative) or as quantified concentrations - cannot be compared between different techniques, and thus findings cannot be extrapolated from one assay to the other. This is seemingly because these represent principally different outcomes, e.g. binding, function, neutralization. Even quantifications obtained by subtypes of the same assay cannot be compared due to different influence of external factors. Altogether, these observations implicate that methodological and clinical validation is a prerequisite when using an assay as basis for TMD-based treatment strategies. Further, that anti-TNF drug and anti-drug Ab values to support therapeutic decisions need to be established for each type of assay. Finally, if TDM-based strategies are used for prospective therapeutic guidance, those patients who are classified differently by different assays will receive principally different interventions - and this is likely to have profound consequences for individual patients' outcomes.

# Accommodation of TDM-related biases

Knowledgeable interpretation of measurements of anti-TNF drug and anti-drug Abs should take the clinical context into account as similar test results can derive from profoundly different clinical scenarios. This is exemplified in Figures 10 and 11, and will be addressed in the sections below. Interpretation also mandates recognition of the interplay between the technical profile of the assay used including its methodological limitations, the timing of measurement during the course of treatment, and the clinical context. Therefore, test results should not stand alone but rather be considered as a tool for evaluation of PK and PD related issues during anti-TNF therapy. To accommodate for biases related to anti-drug Ab detection, interpretation of the clinical relevance of detected anti-drug Abs should include assessment of the concom-

itant anti-TNF drug level. Furthermore, in case of discrepancies between clinical presentation and test results, measurements of the same sample by different types of anti-drug Ab assays can provide additional information not obtained by single assay assessments; for example by assessment of neutralizing anti-drug Abs by a functional assay and the sum of neutralizing and nonneutralizing anti-drug Abs by a drug tolerant assay. Finally, assessments may favorably be repeated during the course of treatment to monitor the evolution of anti-TNF drug and anti-drug Abs over time. This is relevant because the PK and PD of TNFinhibitors are dynamic and change over time. Also, this can be done to accommodate for temporal biases related to the timing of sampling during the course of treatment. Repeated assessments over time is particularly relevant in the event of simultaneous detection of anti-TNF drug and anti-drug Abs, or at suspected anti-drug Ab transiency, as the clinical relevance of these phenomena is not well defined.

# FIGURE 10: TDM-BASED TREATMENT ALGORITHM FOR HAN-DLING PATIENTS WITH ANTI-TNF TREATMENT FAILURE

|                                      | Detectable anti-drug Abs  | Undetectable anti-drug Abs  |
|--------------------------------------|---|---|
| Sub-therapeutic anti-TNF drug levels | Insufficient bioavailability of<br>the anti-TNF agent due to<br>induced immunogenicity<br>with functional anti-drug<br>Abs that increase drug<br>clearance.   | Insufficient anti-TNF drug<br>bioavailability due to non-<br>immune mediated pharma-<br>cokinetic issues. Alterna-<br>tively, potential compliance<br>issues in case of self-<br>administration.  |
|                                      | Change to different TNF-<br>inhibitor.  | Intensify the treatment<br>regimen of the currently<br>used TNF-inhibitor.  |
| Therapeutic anti-TNF drug levels     | Consider:<br>(A) Non-functional anti-<br>drug Abs.<br>(B) False positive anti-drug<br>Ab test.  | Consider:<br>(A) Pharmacodynamic is-<br>sues where inhibition of<br>TNF-α is ineffective due to<br>non-TNF driven disease.<br>(B) Symptoms caused by<br>other problems than active<br>IBD.  |
|                                      | Repeat assessments of anti-<br>TNF drug and anti-drug Abs<br>over time and/or with<br>functional assay and handle<br>accordingly.<br>If unchanged results, then<br>the condition is considered<br>to be caused by a pharma-<br>codynamic problem. | TNF-inhibitors not effective<br>and are discontinued. Re-<br>view of clinical condition:<br>If symptoms originate from<br>IBD related inflammation,<br>then use drug(s) with other<br>target than TNF-α.<br>If symptoms do not origi-<br>nate from IBD related in-<br>flammation, then treat the<br>underlying problem. |

# Figure 10 legend:

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# FIGURE 11: TDM-BASED TREATMENT ALGORITHM FOR HAN-DLING PATIENTS IN CLINICAL REMISSION DURING ANTI-TNF THERAPY

|                          | Detectable anti-drug Abs  | Undetectable anti-drug Abs   |
|--------------------------|---|--|
| drug levels              | Remission is independent<br>of inhibition of TNF-α.   | Remission is independent of inhibition of TNF-α.   |
| Sub-therapeutic anti-TNF | Discontinue anti-TNF therapy.   | Discontinue anti-TNF thera-<br>py.   |
| TNF drug levels          | Consider:<br>(A) Non-<br>functional/transient anti-<br>drug Abs.<br>(B) False positive anti-drug<br>Ab test.                      | Anti-TNF drug induced remission.   |
| Therapeutic anti-        | Repeat assessments of<br>anti-TNF drug and anti-<br>drug Abs over time and/or<br>with functional assay and<br>handle accordingly. | Continue anti-TNF therapy<br>with current agent. Consider<br>if anti-TNF therapy can be<br>discontinued in selected<br>patients with favorable prog-<br>nosis based on individual risk-<br>benefit evaluation. |

# Figure 11 legend:

It is recommended in all situations to repeat assessments of anti-TNF drug and anti-drug Abs over time and/or with functional assay in order to accommodate for technical and temporal biases. In addition, to assess disease activity status by objective measures by e.g. endoscopy before considering the proposed interventions.

# PREDICTORS OF OUTCOME OF ANTI-TNF THERAPY CLINICAL PREDICTORS

Clinical features relatively consistently associated with better outcomes of anti-TNF therapy include a shorter duration of disease, younger age, non-smoking, normal body mass index, colonic Crohn's disease, inflammatory as opposed to fistulizing Crohn's disease, and mucosal healing attained during anti-TNF therapy (I).[20;31;33;49;50;52;79;95;256-271] Variables associated with relapse of active IBD during ongoing anti-TNF therapy, as well as with corresponding clinical outcomes when applying an intensified anti-TNF treatment regimen or when switching to another TNF-inhibitor in this situation, are not well defined (I;II;III;IV;V;VI;VII).[74;78;93-96;272-288] Risk factors for disease relapse after cessation of anti-TNF therapy during clinical remission essentially relate to markers of disease severity and activity in the form of e.g. younger age, longer disease duration, previous need for an intensified anti-TNF regimen, recent history of systemic corticosteroids for treatment of IBD, fistulizing Crohn's disease, and lack of mucosal healing (VIII).[51;245;289-296] However, observations regarding anti-TNF discontinuation rely mainly on retrospective cohort studies and data have been inconsistent.[296-298]

With respect to the anti-TNF treatment regimen, scheduled rather than episodic therapy is important for prevention of anti-TNF treatment failure, presumably owing to decreased risk of immunogenicity (I;II;III;IV;VI;VII;VIII).[93;139;170;171;299] Furthermore, although an issue of controversy and with recommendations having swung back and forth over the years, the sum of currently available data support superior efficacy of combination therapy between thiopurines and IFX over monotherapy with either agent and especially, but not exclusively, in patients naïve to both drugs treated relatively early during the course of disease as clearly shown in the SONIC trial (I;II;III;IV;VIII).[17-23;25;28;29; 31;33;34;45;57;70;85;93;244;263;300-304] This effect has not been reproduced in a study involving MTX, and has not been assessed for other TNF-inhibitors.[34] The mechanism of increased efficacy of combination therapy remains unknown but is speculated to rely on an additive or synergistic effect between the agents resulting in reduction of systemic inflammation and/or inhibition of immunogenicity of the TNF-inhibitor, ultimately leading to higher anti-TNF drug exposure and thus more efficacious immunosuppressive therapy (Table I).[132;171;175;176] Coherently, combination therapy has independently been associated with higher anti-TNF drug levels and lower levels of anti-drug Abs (II;III;VI;VII).[31;33;34;117;132;170;171;175;176;213;305;306] Further, in patients on concomitant thiopurine treatment, 6tioguanine nucleotide (TGN) levels ≥125 pmol/8 x 108 red blood cells has been associated with maximal effect in securing higher IFX levels during combination therapy. [307] However, as previously mentioned it appears that combination therapy confers little protection against anti-TNF treatment failure beyond one year for anti-TNF naïve patients, and beyond 6 months for previous thiopurine non-responsive patients.[176;242-244;304] As anti-TNF therapy cannot be used to bridge to conventional immunosuppressives, this notion along with safety concerns related to combination therapy primarily in the form of potentially increased risk of infections and malignancies, have led to yet unresolved considerations on whether the conventional immunosuppressive agent favorably can be discontinued in patients in longterm sustained remission on combination therapy (VIII).[28;92;109;243;291;298;308-311]

# SEROLOGICAL PREDICTORS

Presence of inflammatory active IBD is a prerequisite for effective anti-TNF therapy.[17;18;20;23;26;27] Coherently, high baseline Creactive protein (CRP) at treatment initiation as well as early normalization of CRP during anti-TNF therapy has generally been associated with favorable treatment outcomes, albeit not in all studies (I;II;III;IV;V;VI;VII).[31;73;78;195;212;239;241;265;270; 312-315] However, it is unknown whether elevated CRP is truly predictive of response to anti-TNF therapy (Table I); or rather is a confounder reflecting proper selection of patients with active intestinal inflammation as opposed to patients with predominantly non-inflammatory symptoms. Low levels of CRP and low total white blood cell count at cessation of anti-TNF therapy among patients in clinical remission, as well as low fecal calprotectin levels, have been associated with reduced risk of relapse (VIII).[291;295;296;316-318]

Among other potential serologic markers, low albumin levels and positivity of anti-neutrophil cytoplasmic antibody (ANCA) have independently been associated with treatment failure in IBD patients and ulcerative colitis patients, respectively (Table I).[128;129;155;212;319;320] Furthermore, pre-existing lowaffinity IgG Abs cross-reacting with the Fab region of IFX and present prior to initiation of IFX therapy in some patients, has been found to be associated with decreased efficacy and increased risk of infusion reactions.[321] Pre-existing anti-IFX Fab Abs are believed to be components of the natural Ab population, or to originate from adaptive immune responses to environmental antigens or homologous biotherapies, or to comprise antiallotype Abs induced by maternal-fetal IFX transfer followed by recognition of the G1m17 allotype contained in the Fab part of IFX.[322-329]

PREDICTORS RELATED TO THE INFLAMMATORY PHENOTYPE Preliminary gene and cytokine expression studies indicate that patients with a predominantly TNF- $\alpha$  driven disease phenotype exhibit an overall more favorable response to anti-TNF therapy than patients with more pronounced involvement of alternative, non-TNF driven disease pathways.[154;194-204] This has been supported by observations by confocal laser endomicroscopy showing that high levels of membrane-bound TNF expressed by intestinal immune cells is predictive of a favorable outcome of anti-TNF therapy.[330] In addition, observations that primary and secondary anti-TNF treatment failure is relatively often accompanied by high anti-TNF drug levels thus indicating adequate suppression of TNF- $\alpha$  mediated inflammatory processes, support lack of treatment effect to be caused by TNF-independent disease processes either primarily or as a result of redundancy induced by anti-TNF therapy in subgroups of patients (II;III;IV;V;VI;VII).[88;91; 134-136;138;139;143;144] Unfortunately, it is not currently possible to differentiate between TNF- and non-TNF driven inflammatory phenotypes in individual patients. Furthermore, detailed characterization of predominantly or exclusively non-TNF driven inflammatory disease phenotypes to identify alternative and more appropriate biologic targets on an individual patient basis has not yet been carried out. A single study reported that normalization of mucosal gene expression of TNF and/or IL17A is associated with long-term remission after discontinuation of anti-TNF therapy, suggesting that normalization of the intestinal inflammatory phenotype could support withdrawal of TNF-inhibitors.[331]

# GENETIC PREDICTORS

The fact that therapeutic responses to TNF-inhibitors are relatively stable and with similar distributions between different anti-TNF agents, and that patients with different response types have distinct gene expression profiles, suggests that genetic variation at least to some extent characterize subgroups of patients with disparate anti-TNF efficacy profiles.[113;196;197;200] Pharmacogenomic studies have focused mainly on genes encoding proteins related to the TNF/TNF-receptor system and apoptosis.[262;332]

Polymorphisms in the gene encoding TNF- $\alpha$  has been variably associated with anti-TNF treatment outcomes.[195;333;334] TNF- $\alpha$  binds transmembrane cell surface TNF receptor superfamily (TNFRSF) 1A and 1B (TNF-R1 and R2) and via NF- $\kappa$ B activation modulate complex immunoinflammatory processes which can affect cell proliferation, differentiation, survival, and apoptosis depending on cell types and co-activating danger signals.[335-337] Genetic variations in the form of selected single nucleotide polymorphisms (SNPs) in the genes encoding TNFRSF 1A and 1B in relation to outcome of IFX therapy in patients with Crohn's disease was explored in study I. A relatively strong association was observed between TNFRSF1B, rs976881 minor allele carriage and loss of response to IFX maintenance therapy as well as on the biologic response to IFX defined by CRP levels. This SNP has not vet been assessed by others. In addition, TNFRSF1B, rs1061622 minor allele carriage was associated with a beneficial outcome of both IFX induction and maintenance therapy in study I, but with conflicting data reported in the literature.[334;338;339] This discrepancy may relate to limitations of study I such as a limited sample size, lack of correction for multiple comparisons, and lack of a confirmatory cohort. However, it could also be a consequence of genetic heterogeneity between distinct patient populations exemplified by the absence of CARD15 mutations in some ethnic groups.[340] TNFRSF1B expression profiles from patients with Crohn's disease extracted from microarray data showed differences in expression levels of TNFRSF1B between responders and non-responders to IFX both during treatment initiation and in relation to individual IFX infusions (I).[197] Thus, these observations indicate that genetic variations in TNFRSF1B, but not TNFRSF1A, influence the clinical outcome of IFX therapy in subgroups of patients. [334;338;339] However, findings need to be validated and extended to larger and independent cohorts.

Anti-TNF agents act in part through induction of apoptosis, and genetic variations in apoptosis-related genes of TNF superfamilily Fas ligand (FASLG) has thus not surprisingly been associated with effectiveness of IFX therapy (I).[341;342] Accordingly, carriers of the minor allele of FASLG, rs73110 had substantially increased risk of acute infusion reactions to IFX (I). Of note, TNFRSF1B, rs1061622 plays a role in the regulation of TNF-induced apoptosis possibly linking findings on this SNP presented above, to a puta-tive functional mode of action via apoptosis pathways (I).[343]

A few studies have noted an association between immunogenicity to TNF-inhibitors and genetic variations in genes encoding e.g. the human leukocyte antigen (HLA) system.[324;344-346] If these findings holds true, then this would provide a potential theoretical explanation for the observations presented in study VI that some patients are more prone to develop antibodies against TNFinhibitors than others. A single study of limited sample size reported that genetic variations in genes encoding IBD5 or NOD2/CARD15 did not associate with the clinical outcome after discontinuation of IFX therapy in Crohn's disease patients in remission.[347] Finally, influence of genetic variation on factors that potentially affect anti-TNF clearance other than anti-drug Abs are not fully understood, e.g. related to the FcRn pathway, albumin, and Fcy receptors (Table I).[152;153;162;348;349]

# PERSONALIZED ANTI-TNF THERAPY CONSIDERING PATIENT, DIS-EASE, AND TREATMENT CHARACTERISTICS

Despite identification of a large number of patient-, disease-, and treatment related variables that are associated with different clinical outcomes of anti-TNF therapy, a personalized anti-TNF treatment approach comprising a priori selection of appropriate candidate patients, optimal interventions at insufficient effect or manifest treatment failure, and identification of patients in remis-

sion in whom anti-TNF therapy can favorably be discontinued, cannot presently be performed on an individual patient basis considering these factors alone. Although findings have been promising, they are inconsistent and require further validation, reproduction, and simplification before they can be incorporated into a risk stratification model and implemented in clinical practice. Until then, knowledgeable interpretation of relevant variables associated with anti-TNF treatment outcomes, and proactive use of corresponding appropriate interventions, can be used by physicians to improve the likelihood of favorable anti-TNF treatment outcomes and reduce the risk of treatment failures. Taken together, these observations stress a need for alternative approaches to improve anti-TNF treatment outcomes on an individual patient basis, for example by TDM-based strategies which will be detailed below.

# ANTI-TNF THERAPY OPTIMIZATION BY TDM-BASED STRATEGIES

Following initiation of anti-TNF therapy, different strategies that incorporate TDM in the clinical management of individual patients have been proposed. These strategies relate primarily to three clinical scenarios comprising manifest anti-TNF treatment failure, prevention of anti-TNF treatment failure, and discontinuation of anti-TNF therapy during remission.

# MANAGEMENT OF ANTI-TNF TREATMENT FAILURE BY TDM-BASED STRATEGIES

#### Conventional management of anti-TNF treatment failure

Up to half of patients with favorable clinical response to anti-TNF maintenance therapy eventually lose effect and experience flare of disease. [43;88] Most studies define loss of response, also referred to as secondary non-response, according to the treating physician's global evaluation and usually by an accompanying need for medical or surgical interventions, and preferably supported by a validated disease activity scoring system, e.g. a Crohn's Disease Activity Index (CDAI) score >150 point and with a clinically relevant increase by  $\geq$ 70 points.[88;88;94;280;287] The true magnitude of this problem is hard to assess because employed definitions have varied, and with variable time-points of outcome reportings and diverse follow-up times. Notwithstanding, systematic reviews have estimated that secondary treatment failure defined by the need for dose intensification occurs in nearly half of anti-TNF treated Crohn's disease patients, and at an annual risk of approximately 13% for IFX and 25% for ADL.[93;95] However, the incidence is not constant being higher in the first year and subsequently leveling out.[94]

Current guidelines and clinical practice suggest intensifying the anti-TNF regimen in case of secondary treatment failure.[18-25] This 'empiric' strategy is based on the assumption that it is preferable to completely exhaust treatment options with the current anti-TNF agent before discontinuing its use, and combined with uncontrolled observations from clinical trials and cohort studies showing that clinical effect can be restored in the short term in more than half of the patients. [20;45;70;93;95;96;272;273;275-280;282;284;286;287;299;350] A comparable short term outcome after IFX dose intensification was observed in studies II, III, and IV. A decision analysis model study based on clinical trial data found marginally higher quality-adjusted life years attained by IFX intensification than by switching to a standard ADL regimen at treatment failure; however, this difference came at considerable costs. [351] PK modelling studies have yielded conflicting results regarding the optimal anti-TNF dose intensification strategy.[160;352] However, available data does not support a clinical relevant difference between treatment intensification by increased dosing or increased frequency of administrations.[240;275;277]

Even though a minority of patients is later able to de-escalate and go back to the standard anti-TNF regimen, a substantial proportion does not respond to anti-TNF treatment intensification or lose effect of the intensified regimen over time ultimately leading to complete treatment failure and discontinuation of therapy.[93-96;276;278;279;282;284;286;287] Switching within drug class to a different TNF-inhibitor is generally preferred in this situation and recaptures response at overall similar rates as dose intensification; however, with decreased efficacy as compared with outcome of treatment with the primary anti-TNF agent, and once again with notable risk of losing response over time. [20;74;78;80; 94;273;274;281;283;285;288;353;354] Curiously, elective switching of anti-TNF agent from IFX to ADL in IBD patients in remission results in worsening of the disease as compared to continued IFX therapy.[355;356] Switching out of class to a biologic agent with a different mode of action has recently become an alternative option after treatment failure on one or more TNF-inhibitors, or even as the primary choice of biologic therapy, as the leukocyte migration inhibitor, vedolizumab which selectively inhibits the trafficking of gut-homing CD4+ T lymphocytes to the gut by targeting the  $\alpha 4\beta 7$  integrin, has recently been approved. [357-359] However, effectiveness of vedolizumab is lower in patients with previous anti-TNF treatment failure than in biologically naïve patients, and data are still too scarce to allow for conclusions on which specific patients may derive the most benefit from treatment with this new agent and its exact positioning in clinical practice is yet to be defined. [360;361] Patients with primary anti-TNF treatment failure are generally handled by similar empiric principles as patients with secondary failure although data are limited and guidelines do not give specific recommendations for handling this subgroup.[18-25] Taken as a whole, no RCT have compared efficacy and cost-effectiveness of these empiric treatment approaches at anti-TNF treatment failure.

### TDM-based management of anti-TNF treatment failure

My research group has put forward an alternative to the 'empiric' strategy at anti-TNF treatment failure.[99] This is a TDM-based strategy exploiting measurements of anti-TNF drug levels and anti-drug Abs at the time of manifest treatment failure to identify proposed underlying PK and PD related mechanisms for failure in each individual patient, and to prospectively guide clinical interventions accordingly. As outlined in Figure 10, this TDM-based strategy integrates current knowledge on the PK and PD of TNFinhibitors into an algorithm which operates with principally distinct mechanisms for anti-TNF treatment failure defined by therapeutic or sub-therapeutic circulating anti-TNF drug trough levels, and detectable or undetectable anti-drug Abs at the same time. Hence, in case of manifest treatment failure at sub-therapeutic drug levels, it is speculated that treatment failure is caused by a PK problem with insufficient drug bioavailability to adequately suppress TNF- $\alpha$  mediated inflammatory disease activity thus resulting in flare of disease. The treatment should therefore restore sufficient inhibition of TNF-a. In order to optimally do so, it is suggested to intensify the treatment regimen of the current TNF-inhibitor if anti-drug Abs are absent, as these patients are likely to suffer from a non-immune mediated PK problem – given that lack of adherence has been ruled out for self-administered anti-TNF agents (Table I).[362;363] Conversely, an immunemediated PK problem is assumed if anti-drug Abs are detected together with sub-therapeutic drug levels. As there is little or no cross-reactivity between anti-drug Abs against currently used TNF-inhibitors, switching to a different TNF-inhibitor is advocated in this situation (VI).[146;165;193]

Anti-TNF treatment failure occurring at therapeutic drug levels is suspected to arise from a PD mechanism due to predominantly non-TNF driven inflammatory disease pathways (Figure 10). Here, TNF-inhibitors are considered ineffective and should be discontinued, and the anti-inflammatory treatment should preferably comprise drugs with other targets than TNF- $\alpha$ . These patients were in study II handled by optimization of conventional therapies as no other biologic agents were approved for treatment of IBD at time of these studies. However, non-inflammatory conditions resembling symptoms of active disease such as strictures, bile acid malabsorption, and irritable bowel syndrome (IBS), as well as non-IBD related inflammation due to e.g. infection, vasculitis, ischemia etc., will also likely present at therapeutic drug levels. These complications should therefore be ruled out at this stage, by thorough evaluation of the clinical condition combined with biochemical, endoscopic, and imaging techniques. Patients with symptoms resembling active disease but without presence of IBD related inflammation were in study II handled by anti-TNF discontinuation and treatment of the underlying problem according to clinical practice (Figure 10).

Several groups have supported this TDM-based concept for handling IBD patients with anti-TNF treatment failure, and some have proposed slightly modified versions of the algorithm primarily related to the timing of objective disease activity assessment prior to measuring anti-TNF drug and anti-drug Abs, and the timing of anti-drug Ab assessment after quantification of the drug level due to drug sensitivity of the assay. [87;90;94;110;116;117; 139;149;158;364;365]

# **Optimal management of anti-TNF treatment failure**

Study II is hitherto the only RCT having compared empiric anti-TNF treatment intensification and personalized TDM-based interventions at anti-TNF treatment failure. In this Danish multicenter study, 69 Crohn's disease patients with symptomatic IFX treatment failure during standard maintenance therapy, defined by CDAI ≥220 or ≥1 draining perianal fistula, were equally randomized to IFX treatment intensification with infusions of 5 mg/kg every 4 weeks, or algorithm-defined interventions as outlined in Figure 10. Co-primary endpoints assessed after 12 weeks comprised clinical and economic outcomes. Clinical response rates were comparable between the two strategies both in the intention-to-treat (ITT) (53% vs. 58%) and per-protocol (PP) populations (53% vs. 47%). Although formal non-inferiority could not be declared in the PP population, point estimates of the difference between treatments were very close to zero both in the ITT and PP populations, indicating that the TDM-based strategy did not result in inferior efficacy compared with empiric dose intensification. Furthermore, several biases and confounders may have disfavored the effectiveness of the algorithm, e.g. the modest cohort size and premature study termination due to recruitment problems, the multiplicity of interventions taken, and sizable nonadherence of physicians to the TDM-based strategy in situations where anti-TNF therapy should have been discontinued and combined with the majority of patients failing IFX in the presence of therapeutic drug levels thus should have discontinued anti-TNF therapy according to the protocol. Along this line, and due to the

small number of patients with a proposed underlying PK mechanism for IFX treatment failure, this study did not allow for meaningful comparisons on the clinical outcomes between empiric dose intensification and algorithm defined interventions in these subgroups. However, a trend of inferior effectiveness of IFX intensification was observed in patients with neutralizing anti-IFX Abs detected by RGA as compared to those without (IV).

Nonetheless, the similar clinical outcomes came at substantial cost reductions by the TDM-based strategy comprising 34% in the ITT population and 53% in the PP population. The basis for this difference was lower costs attained by avoiding inappropriate use of anti-TNF drugs. Cost estimates included all costs related to treatment of Crohn's disease including expenses for measuring IFX and anti-IFX Abs, and had high internal validity being based on the Danish National Patient Registry. Cost estimates were robust to price reductions of TNF-inhibitors by up to 7%, and also to variations in administration costs and in patients' weights. Even though exact cost figures cannot be directly extrapolated to other countries, there is no reason to expect fundamentally different results in other healthcare settings, as expenses for biologic agents are substantially higher than all other currently available medical interventions. Furthermore, in a scheduled 20-week follow-up extension of the trial reported in study III, clinical outcomes continued to be similar between the two randomization groups and in all study sub-populations, but with patients handled in accordance with the algorithm having a higher propensity of continuing the same type of treatment after end of trial. In addition, economic superiority of the algorithm was maintained throughout a one-year follow-up period with relatively stable cost-reduction percentages, and with significant cost-reductions by up to 60% in patients persistently treated as defined by the algorithm.

Accumulating data from observational studies have extended our RCT findings. Hence, in line with the results of studies II and III, a simulation model study integrating available clinical trial data from patients with Crohn's disease reported similar clinical outcomes between empiric intensification of the IFX regimen at treatment failure and TDM-based interventions, but again at significantly lower costs assessed after one year. [366] A retrospective study of a mixed IBD cohort comprising 121 Crohn's disease patients and 31 ulcerative colitis patients with partial or complete IFX treatment failure, found superior clinical outcomes when anti-IFX Ab-positive patients were changed to another TNFinhibitor as compared to IFX dose intensification (92% vs. 17%); and superior outcomes of dose intensification at sub-therapeutic IFX levels as compared to switching to another anti-TNF agent (86% vs. 33%). Coherently, a retrospective study of 64 Crohn's disease patients and 26 ulcerative colitis reported significantly lower response rates attained by IFX dose intensification in the presence of persistent anti-IFX Abs (16%) as compared to dose intensification in patients with transient anti-IFX Abs (69%) or without anti-IFX Abs (94%).[240] Similarly, in a retrospective study of 188 IBD patients with IFX treatment failure, anti-IFX Abs was associated with a 90% specificity for failure to regain response by IFX dose intensification, and with substantially shorter duration of sustained regained response among dose intensified anti-IFX Ab-positive than anti-IFX Ab-negative patients.[367] Furthermore, data from across these trials and in line with the observations presented in studies II, III, IV, and V show that patients with the apeutic IFX levels at treatment failure have a very low chance of regaining treatment effect by use of continued

anti-TNF therapy, irrespectively of treatment intensification or change of TNF-inhibitor. Hence, these patients with a presumed PD problem have comparable outcomes when symptomatically treated without use of TNF-inhibitors as compared to continued and intensified anti-TNF therapy (II;III); with preliminary data suggesting superior outcomes when optimized on conventional immunosuppressives or switched out of class to a biologic drug with another therapeutic target than TNF- $\alpha$ .[367;368]

The considerations regarding TDM-based identification of underlying PK and PD related mechanisms and corresponding rational clinical interventions in patients with secondary anti-TNF treatment failure are in principle similar in patients presenting with primary anti-TNF treatment failure. However, data from this subgroup is almost non-existing and special conditions regarding increased drug clearance among these patients may apply as described below.[91] Similar conditions are also expected to apply to all other TNF-inhibitors. Hence, as previously detailed and as observed in studies VI and VII, circulating ADL trough levels and anti-ADL Abs are associated with clinical outcomes of ADL therapy.[135;140;143;144;172;214;369] Congruent findings supporting clinical superiority of TDM-based therapy over empiric treatment at ADL treatment failure has also been reported in a retrospective cohort study comprising 142 IBD patients.[367] Additional support has come from a prospective observational study where 82 IBD patients with secondary ADL treatment failure were first dose intensified on ADL and followed by switching to IFX in case of insufficient ADL effect. [370] Here, patients with a non-immune mediated PK problem had significantly higher remission rates on an intensified ADL regimen (67%) as compared to patients with immune-mediated PK (12%) or PD problems (29%); and switching to IFX therapy was significantly more effective in patients with an immune-mediated PK problem (80%) than in patients with non-immune mediated PK (25%) or PD (7%) problems. Overall comparable findings have also been reported in patients with rheumatoid arthritis.[371-373]

Taken together, the sum of available evidence appears to support a personalized, TDM-based management approach to IBD patients presenting with manifest anti-TNF treatment failure. At treatment failure, a TDM-based strategy has several advantages over an empiric approach as it avoids unnecessary dose intensification, allows timely targeted switching between anti-TNF agents, and directs therapy to other now available therapeutic options, when appropriate. The result is superior clinical outcomes attained at highly reduced treatment costs. Furthermore, as anti-TNF treatment failure has immediate negative impact on patientreported outcomes in the form of HRQOL and productivity measures which is only reversed once disease activity has been brought under control, TDM-based interventions are also likely to minimize patient impairment and indirect disease-related costs by avoiding delay of optimal treatment (IX).[14;16;374;375] Despite resulting in discontinuation of anti-TNF therapy in a majority of patients and lowering direct treatment costs, use of a personalized TDM-based treatment strategy in studies II and III did not negatively influence patient-reported outcomes or indirect costs (IX). The impact of findings regarding the use of a personalized, TDM-based strategy at anti-TNF treatment failure including results presented in this dissertation, has resulted in this approach now being recommended in the newest clinical guidelines for ulcerative colitis in North America.[25] However, it is acknowledged that large RCT including adequately powered comparisons of outcomes in subgroups with different proposed underlying PK

and PD mechanisms for anti-TNF treatment failure, and preferably incorporating an option of switching out of biologic drug class in patients with proposed PD treatment failure, are needed to unequivocally conclude on the clinical superiority of TDM-based interventions at anti-TNF treatment failure in all patient subgroups. Such trials are currently underway (ClinicalTrial.gov: NTC01960426). Furthermore, recent introduction of anti-TNF biosimilars may further reduce cost savings attained by TDMbased management of anti-TNF treatment failures.[376;377] Finally, a number of unresolved issues need clarification as outlined below and explored in studies II through IX.

# TDM-based management of anti-drug Ab positive patients at treatment failure

Because there are not always observable clinical consequences of anti-drug Ab detection, some hold the view that anti-drug Abs are irrelevant, should never be measured, and should not warrant discontinuation of the current anti-TNF agent in case of treatment failure.[136;170;226;227] These assumptions are fostered by the use of assays known to yield false test results.[115;166;205-207;209] Observations that anti-drug Abs are sometimes only transiently present in the circulation and without apparent clinical impact has led others to recommend switching between anti-TNF agents at loss of response only at high-titre anti-drug Abs and sub-therapeutic drug levels; and to apply dose intensification combined with optimized conventional immunosuppressive therapy at low-titre anti-drug Abs (IV;VII).[90;116;149;180;238;240; 251;307;364;367;378] However, rather than an 'all or nothing' phenomenon, the ability of endogenous anti-drug Abs to overcome and saturate the binding of anti-TNF drug and result in treatment failure is a continuous variable dependent on the amount of exogenous anti-TNF drug administered and the titre of the anti-drug Abs (IV;V;VII). Thus, this author suggests discontinuing therapy with the anti-TNF agent in question if anti-drug Abs at any titer are detected repeatedly in the presence of low drug levels and insufficient treatment effect. This is because the existence and nature of anti-drug Ab transiency remains to be clarified, presumed transiency has been shown to sometimes originate from methodological biases, cut-off values to distinguish between persistent and transient anti-drug Abs are unknown, and the concept of immunological tolerance induction to TNFinhibitors is yet speculative (IV;VI;VII).[180;238;240] It is furthermore documented that there is a high risk of treatment failure and hypersensitivity reactions at continued treatment with the same TNF-inhibitor in the presence of anti-drug Abs, and this strategy is also very expensive due to the high costs of intensified anti-TNF treatments needed to overcome anti-drug Abs whereas other TNF-inhibitors are available for use in standard doses (II;III;IV;V;VI;VII).[180;192;193;238;240;366-368;370]

As antibodies against TNF-inhibitors are highly drug specific and rarely cross-react with other anti-TNF agents, switching from one TNF-inhibitor to another is seemingly unproblematic in the presence of anti-drug Abs (II;III;IV;V;VI;VII).[146;165;167;193;218;240; 366-368;370;379] However, study VI revealed that switchers with previous anti-IFX Ab development more often form Abs against ADL than those without, suggesting that a subgroup of patients are prone to develop an Ab response to TNF-inhibitors in general.[380] Preliminary data from gene variation studies previously detailed support this concept.[324;344-346] Although this novel finding does not justify recommendation against switching between TNF-inhibitors in anti-drug Ab positive patients, it does warrant close monitoring for development of antibodies to the new drug (VI;VII).

# TDM-based identification of non-immune mediated PK vs. PD issues for treatment failure

As previously detailed and outlined in Table I, non-immune mediated PK reasons for anti-TNF treatment failure appear to stem primarily from a high inflammatory load resulting in increased drug clearance, and followed by insufficient inhibition of TNF- $\alpha$ mediated inflammatory disease activity. Furthermore, PD reasons for anti-TNF failure seemingly arise from predominantly or exclusively non-TNF-driven inflammatory disease pathways either primarily or due to redundancy induced during ongoing anti-TNF therapy. These conditions are substantiated by observations in study IV and by others that the increase in circulating anti-TNF drug levels during dose intensification at treatment failure is associated with regained clinical response and mucosal healing only in a subgroup of patients (i.e. those with a proposed nonimmune mediated PK problem), while others fail to retrieve effect despite comparable or higher levels of increased TNF inhibition (i.e. those with a proposed PD problem).[240;367;368;381;382] Differentiation between non-immune mediated PK or PD problems for anti-TNF treatment failure is challenging, and the postfactum timing of observations on changes in anti-TNF activity during treatment intensification makes them impractical for prospective clinical guidance.

Association between anti-TNF trough levels and clinical outcomes combined with observations of an anti-TNF drug concentrationeffect relationship reaching a plateau above which response and remission rates do not seem to increase, indicate that anti-TNF drug levels need to be above a specific threshold for the drug to exert its optimal efficacy.[94;99;117;134;136;138;142;241;383] Identification of such a cut-off value would potentially allow for differentiation between therapeutic and sub-therapeutic anti-TNF drug levels and thereby provide support to identify underlying mechanisms for anti-TNF treatment failure including to distinguish between non-immune mediated PK or PD reasons for treatment failure.[94;99;117;138;367;368;370;378] Initial trials applied the LOD of the assay as a pragmatic cut-off value.[132; 133;136;175;368] In contrast, studies II and III applied a cut-off value of 0.5 µg/mL to distinguish between therapeutic and subtherapeutic IFX levels. This value had been established by receiver operating characteristic (ROC) analysis in a retrospective study of 85 Crohn's disease patients by use of the same RIA as applied in studies II, III, and V; and with favorable test characteristics (sensitivity 81%; specificity of 94%; accuracy 90%).[138] Even though the exact numeric value of this very low cut-off resembling the LOD of may assays can indeed be questioned, and despite observations in study IV that some patients with IFX >0.5 µg/mL still responded to an intensified IFX regimen, exploratory analyses demonstrated that findings in studies II and III were relatively robust to changes in the applied IFX cut-off value. As previously detailed, the exact values of anti-TNF drug levels cannot be directly extrapolated from one assay to another (IV;V).[222] Irrespectively, data obtained by ELISA and HMSA indicate that somewhat higher anti-TNF maintenance trough levels are likely to better differentiate between therapeutic or sub-therapeutic levels. Hence, a minimal IFX level of 3 µg/mL, and an ADL level of 5 µg/mL has been proposed to be optimal in patients with Crohn's disease (VI).[140-142;145;241;305;367;370; 384;385] It appears that levels are generally higher in ulcerative colitis likely owing to an overall higher inflammatory burden

(Table I).[136;138;145;383] It has also become clear from these studies that thresholds vary according to use of concomitant immunosuppression and desired outcome measure, e.g. clinical, biochemical, or endoscopical. Furthermore, that threshold levels are likely different during the anti-TNF induction and maintenance phases, and that despite seemingly high anti-TNF drug levels among primary anti-TNF treatment failures, a proportion of these patients may in fact still experience sub-therapeutic drug levels and thus insufficient effect caused by a non-immune mediated PK problem.[91;241;315;365] This is supported by preliminary data that patients with acute severe extensive ulcerative colitis seem to benefit from a more intensified induction strategy than normally applied, suggesting a need for higher drug exposure and thus higher threshold values during the induction phase probably owing to an excessive inflammatory load (Table I).[365;386] Relevant anti-TNF threshold values remain to be defined for all clinical scenarios and subgroups of patients, by all individual TNF-inhibitors, and by all available assays. In light of the above, it is likely that such values vary between individual patients and even intra-individually across time, making cohort studies less valuable.[219] Irrespectively of these limitations, and acknowledging that the diagnostic impact of a TDM-based strategy at anti-TNF treatment failure is not perfect, the vast majority of patients with suspected treatment failure still benefit from a TDM-based approach (II;III;V).[115;367;368;370]

Along this line, it has become clear that in order to maximize diagnostic accuracy and clinical outcomes of TDM-based interventions at anti-TNF treatment failure, it is necessary at an early stage to assess disease status by objective measures. This is because a proportion of patients with therapeutic anti-TNF drug levels presenting with symptoms resembling anti-TNF treatment failure, and with elevated clinical disease activity indices in keeping with active disease, have other causes of symptoms than flare of IBD (II;III;IV;V).[94;367;368] In fact, incongruence between primarily symptom-based disease activity indices (e.g. CDAI, Harvey-Bradshaw Index, Simple Clinical Colitis Activity Index, the partial Mayo Score etc.) and objective measures of inflammation has recently led the U.S. Food and Drug Administration (FDA) to require documentation of efficacy by objective measures as well as by patient reported outcomes when approving new biopharmaceuticals for treatment of IBD (VIII;IX).[67;70;244;387-396] This potential bias may have influenced findings in studies I trough IX, although classifications were generally supported by biochemical markers of disease activity. At the time when study II was carried out, there was an ongoing discussion in the literature of timing of measurements of anti-TNF drug and anti-drug Abs with respect to endoscopic examination. However, it was not routine practice to perform endoscopy in all patients with symptomatic anti-TNF treatment failure at the time of study II. Thus, according to the applied TDM-based algorithm (Figure 10), only patients with therapeutic IFX levels were assessed by endoscopy, and even though a minority of these patients did not have active disease, this is unlikely to have biased the overall study results as a comparable proportion is expected to have had noninflammatory reasons for symptoms of IFX failure in the control arm due to the RCT design.[397;398] In summary, early objective disease assessment is recommended in order to rule out noninflammatory conditions at suspected anti-TNF treatment failure, but the exact timing with respect to TDM-based assessments should rely on clinical judgement and local logistics.

# Accommodation of potential temporal TDM-related biases at anti-TNF treatment failure

Due to the dynamic nature of the PK and PD of TNF-inhibitors, and to accommodate potential biases related to systemic appearance and temporal evolution of anti-drug Abs, repeat combined measurements of anti-TNF drug and anti-drug Abs over time should generally be considered. This is particularly relevant when treatment failure presents at sub-therapeutic drug levels and undetectable anti-drug Abs, as this status can be a precursor for later appearance of anti-drug Abs as well as a consequence of compliance issues (Figure 10).[115] Reassessments across time is also relevant when anti-TNF treatment failure presents at therapeutic drug levels combined with detectable anti-drug Abs. Hence, this status may originate from false positive test results or detection of non-functional anti-drug Abs (II;III;IV;V;VI;VII) (Figure 10).[115;216;219] However, few patients have been reported to present with combined detection of anti-TNF drug and anti-drug Abs, and if test results remain unchanged at later time points, anti-drug Abs should be considered non-functional and treatment failure to be a consequence of a PD problem (II;III;IV;V;VI;VII) (Figure 10).[142;216;367;368] Of note, a potential time lack between observed changes in blood levels of anti-TNF drug and antidrug Abs and corresponding changes in clinical presentation of symptoms and findings should be expected when adjusting the anti-TNF regimen at treatment failure (IV;VII).[146;238]

# Accommodation of potential methodological TDM-related biases at anti-TNF treatment failure

Having established that analytical characteristics of available assays for anti-TNF drug and anti-drug Ab detections differ, study V explored implications of using different assays for personalized, TDM-based clinical guidance at IFX treatment failure. This study revealed that classification of underlying mechanisms for IFX treatment failure defined by the TDM-based algorithm in Figure 10 among patients enrolled in study II using four principally different analytical techniques based on RIA, HMSA, ELISA, and RGA, and using the LOD by each assay to dichotomize sub-therapeutic or therapeutic IFX levels and positive or negative anti-IFX Abs, resulted in a relatively high level of agreements between each pair of assays of 79-94%. Furthermore, these assays did not differ in their abilities to predict clinical outcomes of interventions defined by the TDM-based algorithm outlined in Figure 10. Coherently, the results of studies II and III were found to be robust to classifications obtained by analytical techniques other than the RIA primarily utilized. The findings of study V are limited by a low number of patients including few patients presenting with a PK mechanism for treatment failure, thus limiting the power to compare treatment outcomes of interventions based on different analytical techniques, and rendering an optimal assay for TDMbased interventions yet to be defined.

A related unresolved methodological issue is whether alternative measures of drug exposure such as area under the curve (AUC), mean serum concentrations, or peak drug levels, provide more information of therapeutic relevance allowing for more robust PK and PD analyses, and thus more robust TDM-based strategies, than trough level assessments.[130] Use of trough level measurements originate from a limitation of ELISA to measure antidrug Abs in the presence of drug, and samples have therefore conventionally been obtained immediately prior to the next drug administration when drug levels are lowest within a therapeutic cycle. However, development of assays that detects anti-drug Abs in the presence of drug, as well as assays that measure the functional effects of anti-TNF drug and anti-drug Abs, has challenged the use of trough samplings.[130;137;221] In addition, assessment of anti-TNF drug exposure in alternative compartments to serum has also been suggested to contribute to improved PK-PD models, e.g. in intestinal biopsy specimens, by in vivo visualization of intestinal anti-TNF drug binding to TNF- $\alpha$ , or by assessment of fecal anti-TNF drug loss.[161;203;330;399]

# PREVENTION OF ANTI-TNF TREATMENT FAILURE BY TDM-BASED STRATEGIES

In addition to aiding rational handling of patients with manifest anti-TNF treatment failure, a TDM-based strategy has also been proposed to aid prevention of treatment failure among patients with stable clinical responses during ongoing anti-TNF therapy.[364;378;400;401] The basis derives from previously outlined data acknowledging an anti-TNF drug exposure-response relationship implicating that low blood drug levels are associated with low efficacy and treatment failure, and combined with observations that even among patients with apparent favorable responses to TNF-inhibitors, low drug levels during the induction phase or early in the maintenance phase are associated with later antidrug Ab formation as well as with later treatment failure during the course of anti-TNF treatment.[132;136;172;175;216;240;241; 369;381;383;384;402-404] This indicates, that the standard dose regimen is probably not uniformly optimal for all patients in remission, and it is therefore hypothesized that anti-TNF treatment failure caused by PK issues can be avoided by continuously maintaining drug levels above the lower limit of a therapeutic concentration window. Conversely, that drug concentrations above an upper limit of a therapeutic concentration window will not result in increased efficacy, and that the dosing of patients with such supra-therapeutic drug levels can be lowered to save costs. Of note, very high anti-TNF drug concentrations do not seem to affect the safety of these agents. [45;57;70;71;78;89;405;406] In summary, a proactive TDM-based strategy to prevent anti-TNF treatment failure advocates monitoring trough anti-TNF drug concentrations at regular intervals in order to allow for dose adjustments to secure drug levels continuously within a specific therapeutic window.

A proactive TDM-based strategy to target a therapeutic window for IFX trough levels during maintenance therapy was first explored in a retrospective study of 48 IBD patients in clinical remission.[407] Here, 25% of patients were proactively IFX dose intensified due to low drug levels, and 10% received a less intensive IFX regimen due to high drug levels. Although limited by a small sample size and changes during the course of study in both the IFX target range (from LOD to 5-10  $\mu$ g/mL) and in the analytical techniques utilized (from ELISA to HMSA), patients receiving a proactive TDM-based IFX treatment regimen had a higher probability of remaining on IFX than conventionally treated patients (90% vs. 69%), they remained on IFX for a longer duration of time, and they had a non-significant trend of lower risk of anti-IFX Ab formation.

Next, a single-center RCT in 263 enrolled IBD patients with stable clinical responses investigated if dosing of IFX defined by a proactive TDM-based strategy where IFX trough levels were measured prior to each infusion and adjusted accordingly to stay within a target trough concentration of 3-7  $\mu$ g/mL, was superior to clinically based dosing of IFX for achieving remission.[408] In the first phase of this study, all included patients were dose optimized to

IFX trough levels of 3-7 µg/mL. This intervention resulted in a significantly higher proportion of patients in clinical remission in the subgroup with initial IFX levels <3 μg/mL (65% vs. 88%). In contrast, patients with IFX levels >7 µg/mL had successful dose de-escalation without affecting remission rates, but with a reduction of drug costs of 28%. On average, 2.1 and 1.4 optimizations, respectively, were needed to get the patients within the prespecified therapeutic range. In the second phase of the study, patients were equally randomized to continued treatment according to the proactive TDM-based strategy or, alternatively, to be handled by clinically based dose adjustments. The primary end-point of the study, clinical and biochemical remission at one year after optimization, was similar in both groups (69% vs. 66%). Thus, the study observed no additional benefit in utilizing an ongoing proactive TDM-based strategy after the initial optimization phase. However, patients handled by the proactive TDM-based strategy had significantly fewer disease flares (7% vs. 17%), less frequent undetectable IFX trough levels (RR 3.7), and a non-significant trend of lower risk of immunogenicity (0 vs. 3 patients).

Taken together, these findings indicate that proactive TDM-based anti-TNF dose adjustments in patients with stable clinical responses improve clinical outcomes and reduce immunogenicity in a subgroup of presumably under-dosed patients, and reduce treatment costs without affecting disease control in a subgroup of presumably over-dosed patients. More data on this strategy are obviously needed before introduction in routine clinical practice. For example, studies should evaluate long-term clinical outcomes attained by this strategy in subgroups of presumed under- and over-dosed patients, and assess overall cost-effectiveness taking into account expenses for repeated measurements of drug and anti-drug Abs and dose escalation in patients with proposed subtherapeutic drug levels. Furthermore, an optimal therapeutic window remains to be defined taking into account the diverse scenarios of different disease types, use of concomitant immunosuppression, presence of anti-drug Abs, potential inter- and intraindividual differences in values etc. An ongoing RCT examining early proactive TDM-based IFX optimization applied after the induction phase may help answering some of these questions (ClinicalTrial.gov: NTC01442025).

# DISCONTINUATION OF ANTI-TNF THERAPY DURING REMISSION SUPPORTED BY TDM-BASED STRATEGIES

Discontinuation of anti-TNF therapy after certain treatment goals have been achieved can be considered for multiple reasons such as high costs of therapy, patient's preferences, and logistics related to for example moving to areas with less access.[105;298;409;410] Although generally well tolerated, anti-TNF therapy increase the risk of infectious complications and possibly also the risk of some types of malignancies such as lymphomas, and safety concerns are therefore a challenge.[92;308;309;311;411-415] In addition, it has been speculated if anti-TNF therapy changes the natural course of IBD making it possible to favorably discontinue therapy again.[297] Current guidelines provide no recommendations with respect to stopping anti-TNF therapies.[17-25;49;409]

Study VIII along with other uncontrolled, mainly retrospective observations of patients having discontinued IFX therapy while in clinical remission, and generally having continued monotherapy with a conventional immunosuppressive agent after IFX cessation, have reported roughly half of patients to maintain remission after one year, but with most patients eventually relapsing over time.[109;245;289-293;295;296;316;317] At a first glance, it therefore appears that IBD follows its natural course once anti-TNF therapy is discontinued, as comparable relapse rates have been reported in pivotal population based IBD cohorts prior to introduction of TNF-inhibitors.[416;417] However, a subgroup of up to one third of patients presumably characterized by clinical, biochemical, and endoscopical remission seem to enjoy a longlasting favorable prognosis without relapse after discontinuation of IFX therapy as observed in study VIII and by others.[245;290;291;293;295;296] Whether this is because interruption of IFX have coincided with the naturally occurring phase of disease quiescence, or is a consequence of potential reversibility of the immune system with normalization of immune homeostasis remains to be explored. [297] The latter has been supported by observations in rheumatoid arthritis on the potential reversibility of immune dysregulation by modulation of regulatory T cells if effective anti-TNF therapy is applied early during the course of disease; along with findings in IBD that a profound drug-free remission after discontinuation of IFX may be achieved in the case of a short duration of disease from diagnosis to start of anti-TNF therapy, and that longer disease duration is associated with increased risk of relapse after discontinuation of IFX (VIII).[245;297]

The optimal anti-TNF withdrawal strategy including time spent in remission before stopping anti-TNF treatment, and the optimal treatment following its discontinuation, has not been clearly defined but RCTs are underway (e.g. ClinicalTrial.gov: NTC02177071) (VIII).[410] However, observations that a notable proportion of patients in remission have undetectable IFX levels suggesting remission to be independent of inhibition of TNF- $\alpha$ , combined with findings that low IFX levels the at time of discontinuation are associated with favorable outcomes, suggest that anti-TNF discontinuation among patients in complete clinical, biochemical, and endoscopical remission may favorably be supported by a TDM-based strategy for example as outlined in Figure 11.[142;245;291;296;305;408;418] My research group is currently exploring this TDM-based approach to support discontinuation of anti-TNF therapy during remission as part of an international, multicenter, sponsor-investigator initiated RCT (ClinicalTrial.gov: NTC01817426).[419] Importantly, in case of relapse after discontinuation of IFX in patients in remission, retreatment with IFX has consistently proven highly effective and with very low risk of adverse events thus offering an attractive treatment option for these patients (VIII).[245;291-293;295;317;402] This phenomenon is likely owing to the nature of this selected subgroup probably being highly anti-TNF responsive and resistant to anti-IFX Ab formation (VI;VII;VIII).

# OTHER TDM-BASED STRATEGIES

Several other TDM-based strategies for anti-TNF treatment optimization have been proposed, but none of these have been sufficiently addressed to be implemented in routine clinical practice. For example, in the context of withdrawal of a conventional immunosuppressive agent during combination therapy, IFX levels >5  $\mu$ g/mL at the time of discontinuation has been associated with low risk of relapse during later anti-TNF monotherapy, suggesting that anti-TNF drug levels can be used as a prognostic tool in this situation.[176] Also, preoperative IFX levels may potentially be used to identify patients at increased risk of complications after IBD surgery.[420] Even though it is generally discouraged to administer episodic anti-TNF therapy, this still occasionally occur either intentionally or due to e.g. pregnancy or surgery.[329] In relation to reintroduction of IFX therapy after a drug pause, anti-IFX Ab formation during the course of reinitation, and particularly after the first IFX administration, is likely to identify patients with increased risk of acute infusion reactions.[191-193;402] Early high IFX levels and absence of anti-IFX Abs at reinitation also seem to identify patients more likely to favorably respond to retreatment thus mirroring previously detailed observations at first anti-TNF treatment series.[402]

# **CONCLUSION & PERSPECTIVES**

Anti-TNF biopharmaceuticals are an important component of the therapeutic arsenal for treatment of IBD offering the ability to induce and maintain clinical remission, heal mucosa, restore HRQOL, and reduce morbidity and disability. Yet, a significant proportion of patients does not respond or lose effect. This dissertation has established that personalized treatment with TNF-inhibitors by the use of a TDM-based algorithm defined by measurements of anti-TNF drug and anti-drug Abs to guide interventions at therapeutic failure can be useful to secure optimal clinical, economic, and patient reported outcomes.

Furthermore, this dissertation has added to the understanding of underlying PK and PD mechanisms for anti-TNF treatment failure and documented the key role of measurements of anti-TNF drug and anti-drug Abs to elucidate these. However, insights obtained as part of the present studies have also pointed out some of the challenges related to application of a TDM-guided treatment strategy at therapeutic failure, for example related to classification of therapeutic vs. sub-therapeutic anti-TNF drug levels to discriminate between PK and PD reasons for treatment failure, risk of misinterpretation of non-inflammatory symptoms resembling anti-TNF treatment failure to be caused by a PD mechanism, and the clinical implications of positive anti-drug Ab test results both during continued treatment with the current anti-TNF agent or when switching to a second anti-TNF agent. Future research is warranted to clarify these issues, and to refine personalized treatment strategies at anti-TNF failure taking into account the personalized TDM-based treatment algorithm and preferably combined with relevant patient-, disease-, and treatment related characteristics integrated into one single model. Additional challenges for the future are to expand the findings of this dissertation to patients with primary anti-TNF treatment failure, to patients with ulcerative colitis failing anti-TNF therapy, and to TNFinhibitors other than IFX.

In the rapidly evolving area of personalized anti-TNF therapy in IBD, this dissertation has laid a foundation for individually tailored anti-TNF therapy by TDM-based strategies involving other clinical scenarios than treatment failure, for example in the context of prevention of anti-TNF treatment failure during ongoing therapy. Along this line, observations presented here and by others that anti-TNF drug withdrawal during remission may be achievable in selected subgroups, led to the proposal of a novel TDM-based algorithm to support decisions on drug withdrawal. Collectively, TDM-based treatment strategies for clinical situations other than treatment failure need to be explored in future studies before they can be introduced in routine clinical practice. Although a large number of variables related to specific characteristics of individual patients, their disease and the anti-TNF treatment regimen, including novel variables presented in this dissertation, have been identified as potential prognostic markers of different clinical outcome types of anti-TNF therapy, this dissertation supports that personalized anti-TNF therapy cannot at this time be performed only on the basis of these factors. However, management decisions that integrate knowledge regarding these factors can aid to improve the overall benefit-risk ratio of anti-TNF treatment outcomes in individual patients. Furthermore, improved understanding of how these multiple variables influence PK and PD of TNF-inhibitors and lead to variable clinical outcomes has great potential to optimize the care of individual patients with IBD, for example by use of models that tailor dosing regimens in real time taking all relevant variables into account to secure sustained optimal drug exposure in a given patient.

Appreciating that TDM-based strategies as of now comprise a crucial component of personalized anti-TNF therapy, novel insights presented in this dissertation regarding characteristics and comparability of principally different analytical techniques for quantification of anti-TNF drug and anti-drug Abs along with identification of potential technical, temporal, and methodological TDM-related biases and corresponding measures to address and avoid these, provide important practical support for interpretation and implementation of personalized, TDM-based anti-TNF treatment strategies in the clinical management of patients with IBD. This dissertation has underlined the need for future research to define optimal, standardized assays that can secure the most benefit of TDM-based treatment strategies. Until then, personalized anti-TNF therapy defined by TDM-based strategies cannot evade potential pitfalls. Thus, although intuitive at first glance, this dissertation stresses that the complexity of TDM-based strategies mandates expert knowledge to fulfill their potential and attain maximal benefits for patients and society.

#### **ENGLISH SUMMARY**

Therapeutic monoclonal antibodies (Abs) targeting the proinflammatory cytokine, TNF- $\alpha$  have revolutionized the treatment of inflammatory bowel disease (IBD), and raised treatment goals from symptom control to maintenance of clinical remission with mucosal healing. However, clinicians are challenged by a significant proportion of patients not responding to TNF-inhibitors or losing effect over time, and by the high costs of these drugs along with their potential side effects. The aim of this dissertation was therefore to examine if anti-TNF treatment outcomes can be improved by tailoring therapy on an individual patient basis by considering relevant prognostic variables. The main finding is that personalized treatment with TNF-inhibitors by use of an algorithm defined by measurements of anti-TNF drug and anti-drug Abs to guide interventions at therapeutic failure can be useful to secure optimal clinical, economic, and patient reported outcomes. Furthermore, the present studies have documented the key role of measurements of anti-TNF drug and anti-drug Abs to elucidate conditions related to pharmacokinetics and pharmacodynamics of these agents in individual patients, and to serve as prognostic markers of anti-TNF treatment outcomes. In addition, knowledge has been provided on how to interpret and integrate measurements of anti-TNF drug and anti-drug Abs in the clinical management of individual IBD patients taking into account potential pitfalls and biases. Hence, the studies forming the basis for this dissertation have yielded novel insights into the technical, tem-

poral, and methodological complexities and challenges related to application of personalized anti-TNF treatment strategies based on measurements of anti-TNF drug and anti-drug Abs, and established measures to proactively address and accommodate these both technically and clinically. Although not yet completely resolved, this dissertation has also laid a foundation for individually tailored anti-TNF therapy by use of algorithms based on measurements of anti-TNF drug and anti-drug Abs involving different clinical scenarios than treatment failure, for example in the context of drug withdrawal among selected subgroups in remission. Finally, this dissertation has demonstrated that personalized anti-TNF therapy cannot at this time be done on the basis of prognostic variables related to specific characteristics of individual patients, their disease and the anti-TNF treatment regimen; but that management decisions integrating knowledge of these factors can aid improving the overall benefit-risk ratio of anti-TNF treatment outcomes in individual patients. In conclusion, this dissertation has brought personalized anti-TNF therapy in IBD from bench to bedside.

# LIST OF ABBREVIATIONS

| Ab     | antibody                             |
|--------|--------------------------------------|
| ADL    | adalimumab                           |
| ANCA   | anti-neutrophil cytoplasmic antibody |
| AUC    | area under the curve                 |
| CDAI   | Crohn's disease activity index       |
| CRP    | C-reactive protein                   |
| ELISA  | enzyme-linked immunosorbent assay    |
| FASLG  | Fas ligand                           |
| FcRn   | human neonatal Fc receptor           |
|        | (Brambell receptor)                  |
| FDA    | Food and Drug Administration         |
| HLA    | human leukocyte antigen              |
| HMSA   | homogeneous mobility shift assay     |
| HPLC   | high pressure liquid chromatography  |
| HRQOL  | health related quality of life       |
| IBD    | inflammatory bowel disease           |
| IBS    | irritable bowel syndrome             |
| IFX    | infliximab                           |
| lg     | immunoglobulin                       |
| ITT    | intention-to-treat                   |
| LOD    | limit of detection                   |
| MTX    | methotrexate                         |
| PD     | pharmacodynamics                     |
| РК     | pharmacokinetic                      |
| PP     | per protocol                         |
| RCT    | randomized controlled trial          |
| RGA    | reporter gene assay                  |
| RIA    | radioimmunoassay                     |
| ROC    | receiver operating characteristic    |
| RR     | relative risk                        |
| SNP    | single nucleotide polymorphism       |
| T1⁄2   | half life                            |
| TDM    | therapeutic drug monitoring          |
| TGN    | tioguanine nucleotide                |
| TNF    | tumor necrosis factor                |
| TNFRSF | TNF receptor superfamily             |

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