REPORT OF THE ECCO WORKSHOP ON ANTI-TNF THERAPY FAILURES IN INFLAMMATORY BOWEL DISEASES: DEFINITIONS, FREQUENCY AND PHARMACOLOGICAL ASPECTS

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Abbreviations used in the manuscript: IBD: inflammatory bowel diseases; CD: Crohn's disease; UC: ulcerative colitis; TNF: tumour-necrosis factor; Abs: monoclonal antibodies; IFX: Infliximab; ADA: Adalimumab; CZP: Certolizumab pegol; PNR: primary non response to anti-TNF agent; LOR: loss of response to anti-TNF agent; CDAI: Crohn's disease activity

index; CRP: C reactive protein; MRI: magnetic resonance imaging; ATI: antibodies to infliximab; ATA: antibodies to adalimumab; IgG: immunoglobulin G; PEG: polyethylene glycol; FcγR: Fc gamma receptor; ELISA: enzyme-linked immunosorbent assays; RIA: radio-immunoassays.

INTRODUCTION

The introduction of drugs directed against tumour-necrosis factor (anti-TNF) has greatly advanced the therapeutic armamentarium for the treatment of inflammatory bowel diseases (IBD). Infliximab (IFX), followed by Adalimumab (ADA) and Certolizumab pegol (CZP) have shown significant efficacy in severe Crohn's disease (CD) refractory to conventional treatments, including immunosuppressive drugs (Hanauer 2002, Colombel 2007, Schreiber 2007). Efficacy for fistulizing CD has also been shown in a placebo-controlled trial with IFX and in a post-hoc analysis of a pivotal trial with ADA (Colombel 2007, Present 1999). This clinical efficacy has been associated with mucosal healing and improvement in quality of life. The efficacy of anti-TNF agents has also been shown to exert a major impact on the outcome of important disease parameters (i.e. a reduction in hospitalizations and surgeries) (Lichtenstein 2005, Feagan 2008). However, some patients do not respond to anti-TNF agents and a significant proportion of responders may lose response over time.

The scientific committee of ECCO has launched the first pathogenesis workshop which focused on this significant clinical problem. The overall objective was to better understand and explore primary non response (PNR) and loss of response (LOR) to anti-TNF agents in IBD.

The outcome of this workshop is presented into two parts. The first manuscript addresses definitions, frequency and pharmacological aspects of anti-TNF therapy failure, including pharmacokinetics of anti-TNF monoclonal antibodies (mAbs) and immune and non-immune mediated clearance of anti-TNF mAbs. The second manuscript focuses on the biological roles of TNF and TNF antagonists, including mechanisms of action of anti-TNF agents, TNF independent inflammatory pathways, and paradoxical inflammation.

DEFINITION AND FREQUENCY OF FAILURES WITH ANTI-TNF MONOCLONAL ANTIBODIES

Primary non response in luminal Crohn's disease

In placebo controlled trials, the rate of no remission at week 4 was 80% with CZP (Sandborn 2007), 67% with IFX (Targan 1997) and 64% with ADA (Hanauer 2006). These numbers were influenced by induction regimen, mainly for ADA. The rate of non response at week 4 was 71% for CZP (Sandborn 2007), 40% for IFX (Targan 1997) and 41% for ADA (Hanauer 2006). The influence of induction regimen for ADA was not statistically significant.

In pivotal placebo-controlled maintenance trials with open label induction, the maximal response rate was observed at week 12 for CZP and ADA and at week 10 for IFX. The rate of no remission at these time points was 73% with CZP (Schreiber 2007), 58 % for IFX (Hanauer 2002) and 50 % with ADA (Abbott data on file) (Colombel 2007). The rate of no response was 64% and 54% with CZP when defined by a 100 points decrease in CDAI and a 70 points decrease, respectively (Schreiber 2007), 29.2% with IFX (defined by a 70 points decrease in CDAI) (Hanauer 2002) and 31% and 21% with ADA when defined by a 100 points decrease and a 70 points decrease, respectively (Abbott data on file) (Colombel 2007). In these trials, the response and remission rates were influenced by disease duration. For example, no response was observed in only 10% of patients having disease duration of less than 1 year as compared to 43% of patients having disease duration greater than 5 years, at week 26 with CZP (Schreiber 2007).

Mucosal healing has been evaluated with IFX therapy: absence of mucosal healing was found in 71.1% at week 10 and 55.6% at week 54 (Rutgeerts 2006).

In strategy trials, absence of remission without steroids reached a very low rate around 25% at week 12 with IFX combined for a few weeks with steroids, with or without immunosuppressive treatment (Feagan commit 2008, Lémann 2006). Co-treatment with

immunosuppressors was shown to decrease non response but only in immunosuppressor-naïve patients (Colombel 2008). There was no clear effect of immunosuppressor co-treatment in cases of immunosuppressor failures (Sandborn 2007, Targan 1997, Hanauer 2006).

In monocentric uncontrolled series, absence of response after induction were constantly lower than in controlled trials and ranged from 40% to 10% only (Schnitzler 2009, Marting 2007, Gonzalez-Lama 2008, Vermeire 2002). In these series, lower non response rates were associated with immunosuppressor co-treatment, younger age, colonic disease, absence of stricture, non smoking and elevated CRP.

Primary non response in fistulising Crohn's disease

Primary non response after induction with Infliximab was 31 % at 14 weeks (Sands 2004). Absence or incomplete closure at the same time point occurred in 52% of patients (Sands 2004). For ADA, data exists only for the 6 months time point, with absent or incomplete closure in 70% of patients (Colombel 2007). Closure based only on clinical evaluation, does not mean definitive healing as illustrated by MRI assessment. After induction therapy with IFX the vast majority of clinical responders (8/11) had persistent inflammatory tracks on MRI (Van Assche 2003). Monocentric experiences and uncontrolled series suggest that the combination of anti-TNF treatment with an appropriate drainage of perianal lesions and antibiotics may decrease non response rates (Hyder 2006, Topstad 2003).

Primary non response in chronic active ulcerative colitis

Only IFX has currently been adequately evaluated in ulcerative colitis. Absence of response after induction at week 8 was around 35% and absence of remission around 65% (Rutgeerts 2005). Absence of mucosal healing after induction was found in 40% of patients (Rutgeerts 2005).

Secondary non response in luminal Crohn's disease

Secondary non response or loss of response to anti TNF agents is defined in those patients who initially respond to anti TNF therapy and subsequently lost the clinical response. Most studies define clinical response as a reduction in CDAI of ≥70 from baseline and clinical remission as CDAI<150. Secondary non responders are therefore those patients not achieving these clinical goals. For IFX, this is defined if occurring after the forth dose (0, 2, 6 and 14 weeks). For ADA, this is defined if occurring after the induction phase which includes three injections in decreasing doses of 160 mg, 80 mg and 40 mg over a period of 4 weeks followed by 40 mg every other week for a total of 6-12 week period (to achieve maximal response). For CZP, loss of efficacy is present after the induction phase which includes three 400 mg doses at 0, 2, and 4 weeks.

Two placebo controlled trials evaluated Infliximab for the maintenance of remission in CD. Clinical response was defined as CDAI reduction ≥70 from baseline and clinical remission was defined as CDAI<150. Rutgeerts et al evaluated patients who initially responded to IFX at week 44 (Rutgeerts 1999). Failure to maintain response was observed in 38% of IFX treated patients. The proportion of patients not in clinical remission at the end of follow-up with IFX was 47%. Hanauer et al evaluated 335 IFX responders (Hanauer 2002). The median time to loss of response was >54 weeks for IFX 5 mg/kg and 10 mg/kg. Loss of response at week 54 was observed in 61% of patients on IFX 5 mg/kg and in 42% of patients on IFX 10 mg/kg. The proportion of patients not in clinical remission at weeks 30 and 54 were 61% and 71% respectively for IFX 5 mg/kg and 55% and 61.6% respectively for IFX 10 mg/kg. Two trials evaluated the secondary non response to Infliximab by assessing the need to intensify the dose and/or frequency of IFX treatment (Regueiro 2007, Schnitzler 2009). Loss of response was observed in 50%-54% of patients in these studies. A recent big cohort of 614 patients receiving IFX was followed up for a median of 55 months (Schnitzler 2009). The

authors reported non response rate of 21.6% at the end of follow-up. Finally, a recent review of the literature by Gisbert and Panes evaluated data from 16 studies (Gisbert 2009). The reported loss of response rates ranged between 11% and 48%. A total of 2236 patients were included in these studies, providing 6284 patient years of follow-up. The mean percentage of patients with loss of response to IFX calculated from these studies was 37%. Since the follow up time varied between these studies, it was suggested by the authors that the risk of losing response to IFX is better expressed as incidence per patient years of follow up. Using this calculation, the loss of response to IFX was 13.1% per patient year.

Two placebo controlled trails evaluated ADA for maintenance of remission in CD. Similar to the IFX trials, clinical response was defined as CDAI reduction ≥70 from baseline and clinical remission was defined as CDAI<150. Colombel et al evaluated patients who initially responded to ADA at week 54 (Colombel 2007). Loss of response was observed in 46% of the patients. The proportion of patients not in clinical remission at weeks 26 and 54 were 60% and 64% respectively for ADA every other week and 53% and 49% respectively for ADA every week. Sandborn et al evaluated Adalimumab responders at week 56 (Sandborn 2007). The proportion of patients not in clinical remission was 21% and 17% respectively for ADA every other week and ADA weekly.

Two placebo controlled trails – PRECISE 1 and 2 evaluated Cetrolizumab for the maintenance of remission in CD (Schreiber 2007, Sandborn 2007). Clinical response was defined as CDAI reduction ≥100 from baseline and clinical remission was defined as CDAI<150. IN the PRECISE 1 trial, the rate of secondary non responders at week 26 was 38%. The rate of clinical non remission at week 26 was 52%. In the PRECISE 2, secondary non response at week 26 occurred in 38% of patients who initially responded to induction therapy. Clinical non remission occurred in 52% of patients.

Secondary non response in fistulising Crohn's disease

One placebo controlled trail evaluated IFX in the treatment of patients with fistulizing CD (Present 1999). Response was defined as reduction in the number of draining fistulas of at least 50% from baseline and remission was defined as the absence of draining fistulas. At week 54, 64% of the patients had loss of response to IFX manifesting as actively draining fistulas.

Secondary non response in chronic active ulcerative colitis

Two placebo controlled trails, ACT 1 and 2, evaluated Infliximab for the maintenance of remission in UC (Rutgeerts 2005). Clinical response was defined as a decrease in the Mayo score of at least 3 points from baseline and clinical remission as a total Mayo score of 2 or less. In the ACT 1 trail, clinical non response at weeks 30 and 54 were 49% and 55% respectively. Clinical non remission at weeks 30 and 54 were 65% and 66% respectively. Lack of mucosal healing was observed in 50% of patients at week 30 and 55% of patients at week 54. In the ACT 2 trail, clinical non response at week 30 was 53% for Infliximab 5 mg/kg and 40% for IFX 10 mg/kg. Clinical non remission at week 30 was 74.4% for Infliximab 5 mg/kg and 64.2% for Infliximab 10 mg/kg. Lack of mucosal healing was observed in 54% and 43% of patients on IFX 5 mg/kg and 10 mg/kg respectively.

Prevention of anti-TNF therapy failure

Published data from referral centers presenting the rates of response to anti-TNF in routine practice have shown higher response rates than in controlled trials reaching 60–90% of response. These data suggest that an appropriate selection of good candidates to anti-TNF therapy give better results. In the SONIC study, patients with active lesions at endoscopy had higher rates of response to IFX and azathioprine (Colombel 2008).

The use of immunomodulators (Azathioprine, 6-Mercaptopurine and Methotrexate), in conjunction with Infliximab has been shown to significantly reduce the proportion of patients with anti TNF antibodies, possibly leading to a more favourable response and reduced need for dose escalation (Gisbert 2009, Maini 1998, Mortimore 2001). More recently, preliminary results from the ongoing SONIC study, demonstrated higher maintenance of remission rates at 6 months in the combination arm of IFX and Azathioprine. Immunomodulators seem to protect against the induction of anti-ADA and anti-CZP antibodies as well (Schreiber 2007, Sandborn 2007). One placebo controlled trial demonstrated that intravenous Hydrocortisone 200 mg administered immediately prior to IFX infusion, significantly reduced antibody formation to Infliximab; 26% versus 42% in the placebo arm (Farrell 2003). It is not clear however, whether this approach imparts long term effects on loss of response.

Results from several studies have demonstrated that regularly scheduled Infliximab infusions are associated with a decreased likelihood of antibody formation. Intermittent therapy may predispose to formation of anti-drug antibodies and increased loss of response (Baert 2003, Hanauer 2004, Rutgeerts 2004). On the other hand, Zabana et al found no difference in LOR between patients receiving scheduled IFX maintenance therapy to those reintroduced to IFX after a period of 4 months of no therapy in patients who received the original 3 infusion induction regimen (15% versus 10% respectively), suggesting this issue needs further evaluation (Zabana 2008).

PHARMACOKINETICS OF ANTI-TNF MABS

Serum half lives vary between the anti-TNF agents, when administered in humans. Etanercept has the shortest half life (4 days) and ADA and Golimumab between 10-20 days. Elimination of therapeutic proteins varies between individuals and is most likely influenced by immunogenicity (anti drug antibodies) and by differential clearance.

The pharmacokinetics of these agents is determined by three basic factors: - 1) the mode of administration (intravenous vs. subcutaneous) - 2) drug's half-life and - 3) peak-to-trough serum concentration. All these factors determine the therapeutic window, introduced as a concept, by Nestorov in 2005 (Nestorov 2005). The therapeutic window concept postulates that a threshold trough serum concentration is required for therapeutic efficacy. On the other hand, supra-therapeutic serum concentration may increase the hazard of infections or malignancy. The importance of a high peak concentration as a consequence of intravenous administration for efficacy and safety of anti TNF agents in CD and UC has not been established. Peak concentrations after IFX infusion are at least 50 times higher than trough concentrations (100-300µg/mL vs. 1-10µg/mL). This ratio is less prominent in subcutaneously administered agents like ADA, CZP and Etanercept. When administered at a dose of 40 mg EOW in patients with rheumatoid arthritis and CD, the trough serum concentrations of ADA range between 4 and 8µg/mL.

The volume of distribution of IFX and ADA is comparable, which means that these molecules spread similarly into body compartments. It is unclear if this also implies that the penetration in different tissues, such as inflamed gut mucosa, is also similar. To our knowledge, distribution data for CZP are not available.

Importance of pharmacokinetics for the efficacy of anti TNF therapy

When recommended doses are used, one can assume that initially adequate trough serum concentration is obtained in most patients and that low initial concentration is not the reason for PNR. However, data testing this hypothesis are scarce. In the original dose ranging induction trial with IFX a dose response association has not been reported (Targan 1997). Similarly in UC patients, IFX was not superior when given at a dose of 10 mg/kg compared to the 5 mg/kg (Rutgeerts 2005). However, in the first dose ranging trial with ADA in CD a

dose/response relation was apparent (Hanauer 2004). Nonetheless, in all these trials the relevance of early trough serum concentration for individual responses was never reported.

Trough serum concentration of therapeutic antibodies is probably more relevant for secondary LOR. The development of anti-drug antibodies is intrinsically linked with the use of therapeutic proteins (Hwang 2005). However, in clinical practice, only antibodies which interfere with drug efficacy (neutralizing antibodies) or instigate adverse events really matter.

Drug trough serum concentration is reliably assessed regardless of anti-drug antibodies and also reflects the degree of drug degradation. Therefore, this concentration may represent a more clinically relevant surrogate marker for LOR. IFX trough serum concentration correlates with the presence of antibodies to IFX (ATIs) and with duration of response, but this correlation is not absolute (Baert 2003, Maser 2006). Also, a decrease in drug levels may be driven by mechanisms other than the induction of anti drug antibodies. For patients with IBD, more relevant than the underlying mechanism of decreased trough serum concentration is their chance of needing accelerated dosing due to secondary LOR. This information may be inferred from clinical trials. However, it is important to note that in the long term trials with IFX, patients increased the dose in case of LOR whereas with ADA, a shortening of dosing interval was used to enhance drug exposure. In the first maintenance trial for luminal CD with IFX, ACCENT 1, 30% of patients treated with 5 mg/kg iv stepped up to the higher dose group of 10 mg/kg after one year because they experienced a disease flare (Hanauer 2002). In the maintenances trials with ADA, CHARM and CLASSIC II, the percentage of patients that shortened their dosing interval to 40mg weekly after one year was 27% and 46% respectively (Colombel 2007, Sandborn 2007). In the long term maintenance trial with IFX for fistulizing CD, ACCENT 2, 25% of patients increased the dose to 10 mg/kg because their fistulas started draining again (Sands 2002).

Treatment optimization in LOR

If despite optimizing the treatment strategy, the efficacy of an anti TNF agent fades in a patient with initial response, treatment flexibility is needed to counteract LOR. The two main strategies available are: (1) increasing drug exposure by shortening the dosing interval or increasing the dose and (2) switching to another drug. To some extent, the therapeutic intervention needs to be tailored to each individual patient.

To justify the first option of dose escalation, we need evidence that low trough serum concentration is associated with LOR and that increasing drug exposure restores efficacy. In the ACCENT 1 trial, increasing the dose from 5 to 10 mg/kg and from 10 to 15 mg/kg restored response in 62% and in 69% of patients respectively (Hanauer 2002). Conversely, in a single center patient cohort in Leuven of 547 patients with CD, 66% (75/108) regained clinical response until the end of follow up after having shortened their dose interval (Schnitzler 2009). Data in patients with IBD and with rheumatoid arthritis suggest that IFX trough serum concentration below 1µg/mL correlate with LOR (Maser 2006, St Clair 2002). In a retrospective cohort of CD patients at the University of Toronto, ATI formation correlated with low trough concentration, CRP and the absence of long term remission (Maser 2006).

In a prospective immunosuppressives withdrawal trial, patients with CD and with low IFX trough serum concentration (below median) had higher CRP values and CDAI scores than those with trough concentration above median (Van Assche 2008). Hence, even if there is no absolute correlation between trough serum concentration, ATIs and the clinical response, increasing drug exposure with an intention to restore trough concentration to therapeutic values is a valuable strategy. Data regarding the influence of trough concentration on therapeutic efficacy has not been released from the controlled trials that led to the market

authorization of ADA and CZP (Hanauer 2006, Colombel 2007, Sandborn 2007, Schrieber 2005, Schreiber 2007, Sandborn 2007). However, in a retrospective cohort of CD patients treated with ADA at the University hospital of Leuven, trough serum concentration was linked to clinical efficacy. More interestingly, in patients who regained clinical response after dose adjustment, the increment of ADA trough serum concentration was higher than in those who failed to restore response (Karmiris 2009). Similar data were already reported with the use of ADA in patients with rheumatoid arthritis (Bartelds 2007).

The strategies of dose escalation have been very different in clinical trials conducted with the different anti TNF agents IFX, ADA and CZP. Therefore, it is impossible to choose between shortening dosing interval and increasing the dose based on clinical trial experience. For ADA the European label suggests dose intensification only by shortening the interval between injections, but for IFX both options are being employed in clinical practice. A post-hoc analysis of the pharmacokinetic data collected in the ATTRACT maintenance trial with IFX in patients with RA, suggests that shortening the interval will lead to higher trough serum concentration than increasing the dose (St Clair 2002).

In case of LOR despite optimization, other therapeutic options, including switching to another anti-TNF is an option. In the GAIN trial, specifically designed to include patients with LOR or intolerant to IFX, remission rates 4 weeks after an induction dose of 160/80mg ADA were lower when compared to those found in an earlier CLASSIC 1 dose finding trial, (Hanauer 2002, Sandborn 2007). This observation needs to be confirmed, but recent clinical trial data with both ADA and CZP indicate that prior exposure to IFX attenuates the response to a second anti-TNF agent. The reason of discontinuation for failure of one or two anti-TNF mAb (PNR, LOR and/or intolerance) does not seem to influence the rate of response to a second or a third anti-TNF (Sandborn 2007, Vermeire Welcome, Allez 2009).

4. IMMUNOGENICITY OF ANTI-TNF MABS

Anti-TNF agents have different degree of humanization

All anti-TNF agents are compounds produced by biotechnology that mimic molecules found in the body, such as proteins and oligonucleotides. All anti-TNF agents commercially available to treat patients with IBD are monoclonal antibodies or antibody fragments. Etanercept (not effective in CD and not evaluated in UC) is a receptor/antibody fragment fusion protein (Sandborn 2001). Due to their molecular nature all these agents need to be parenterally administered. Several strategies have been followed in drug development to improve the efficacy and tolerability of biological agents. Progress in protein engineering has resulted in the elimination of immunogenic non-human peptide sequences from anti-human antibodies, a technique called humanization (Hwang 2005, Tracey 2008). Third generation, humanized antibodies (±95% human) and fourth generation, fully (100%) human antibodies, are usually associated with less immunogenicity as compared to chimeric (75% human) monoclonals such as IFX. Anti-TNF agents currently available differ in their degree of humanization. The chimeric monoclonal IgG1 antibody IFX (Remicade®, Centocor/Schering-Plough), the human monoclonal IgG1 antibody ADA (Humira®, Abbott), and the humanized Fab antibody fragment linked to poly ethylene glycol (PEG) CZP or CDP-870 (Cimzia®, Celltech/UCB), all binding tumor necrosis factor, have shown efficacy in CD and IFX also in UC. Golimumab (CNTO-148), a fully human IgG1 antibody, is being evaluated for its efficacy in CD.

The methods of detection of antibodies against TNF-antagonists vary among different studies. Notably, not only are the techniques for measurement of antibodies different, even the results obtained by the different methods are not reported in a uniform or standardized manner that

would enable comparability and reproducibility across studies. In this review, firstly, the different methods for the detection of ATI and antibodies to ADA (ATA) and secondly, the impact of immunogenicity on the efficacy and side-effects of both drugs will be described.

Antibodies against Infliximab (ATI)

Initial measurements for detecting antibodies against IFX (ATIs) were mostly performed using solid-phase enzyme-linked immunosorbent assays (ELISA). This technique has a major disadvantage because standard detection antibodies (e.g. labeled anti human Fc) used for the detection of anti-drug antibodies, may also cross-react with the Infliximab moiety that comprises the antigen in these particular assays. To overcome this problem, a double antigen format ELISA has been employed by several groups as well as by a commercial facility (Prometheus Laboratories, San-Diego, Ca, USA). In this technique, plated Infliximab serves as the antigen, and Infliximab is used again, in a biotinylated form, in the detection phase of serum antibodies that bound to the plated IFX – i.e. the antigen (Baert 2003, Vermeire 2007). Nevertheless, this method has several limitations: it can detect only bi-valent or poly-valent ATIs, epitope masking in the plated Infliximab may yield false-negative results and the presence of Infliximab in serum may compete with the detection by biotinylated-IFX. In addition, spontaneously occurring anti IgG antibodies (rheumatoid factor) as well as other low-affinity antibodies may non-specifically bind to the adsorbed Infliximab antigen, yielding a false positive assay result (Svenson 2007).

The limitations of the double-antigen ELISA have lead to development of alternative methods. A functional assay assessing the capacity of sensitized patients' serum to neutralize binding of Infliximab to solid-phase TNF was studied (Candon 2006). This method may carries the risk of missing non-neutralizing antibodies.

Fluid-phase assays comprising radio-immunoassays (RIA) were also studied for ATI measurement. In general, fluid phase RIAs recognize ligands with highly conserved conformations and are therefore less influenced by artefacts due to formation of new epitopes or loss of epitopes occurring after fixing of proteins to solid phase matrices. This technique appears to have the capacity to provide a useful correlation with clinical response to Infliximab (Svenson 2007, Ainsworth 2008, Bendtzen 2006, Bendtzen 2009). A further advantage of fluid phase RIAs is that they also detect functionally monovalent ATIs, such as IgG4, which are not measured by bridging ELISA, but nevertheless constitute a significant amount of anti-inflximab antibodies in patients with rheumatoid arthritis (Svenson 2007). On the other hand, fluid-phase RIA technology does not circumvent the interference stemming from the presence of Infliximab in serum and is still limited for detecting only lambda-chain containing anti-Infliximab antibodies, which have been shown to comprise 50% of the total Infliximab-anti-Infliximab immune complexes in serum (Svenson 2007). Other investigators used agarose-immobilized protein A to capture serum immunoglobulins and then measured radioactivity after addition of I125labeled pepsin-treated Infliximab (Wolbink 2006). However, this method cannot overcome the presence of Infliximab in serum, and may also under-detect anti-Infliximab antibodies other than IgG1 and IgG2, as the latter are preferentially captured by protein A.

Antibodies against Adalimumab (ATA)

One method to measure ATAs consists of adding radio-labelled pepsin-digested Adalimumab (i.e. the Fab2 fragment of Adalimumab) to protein A-captured serum immunoglobulines, with subsequent measurement of sepharose-bound radioactivity (Bartelds 2007). Others have measured ATAs using double-antigen ELISA technique, whereby un-labelled ADA serves as the bound antigen, and labelled ADA is employed in the detection phase (van de Putte 2003). A fluid phase RIA has also been developed (Radstake 2008). The read-outs of this technique

were shown to usefully correlate with clinical response to ADA, or lack hereof, in patients with rheumatoid arthritis and, most likely, with IBD. Since all these methods are in essence similar to those used for ATI as described above, they also share similar technical limitations.

Immunogenicity and Infliximab

Allergic reactions

Acute infusion reactions need to be differentiated from delayed reactions. Acute reactions are defined as reactions occurring during or within 2 hours of an infusion. They can be severe or not. Severe reactions are usually defined as reactions necessitating stopping the infusion due to significant dyspnoea or drop in blood pressure. Mild to moderate acute reactions may include fever, slight decrease in blood pressure, erythema, itching, or shiver.

Delayed reactions occur 2 days to 2 weeks after reinfusion of IFX. The symptoms can be quite severe and usually last 3-5 days. Delayed reactions are usually attributed to serum sickness like reactions. Possible symptoms include a cluster of features (generalized stiffness, myalgias, arthralgias, fever, and/or rash).

The main hypothesis behind these allergic reactions, acute or delayed and severe or not, is that they are related to some form of immunogenicity against IFX. However this has not been adequately studied and the only biological marker available to assess immunization against the drug are the so-called antibodies to IFX (ATI; formerly called human anti-chimeric antibodies or HACAs).

Clinical relevance of immunogenicity and Infliximab

In all registration studies with IFX, ATIs have been detected in 4 to 38% of patients (Hanauer 2004, Sands 2004). In the early post marketing clinical experience when IFX was used on

demand with and without concomitant immunosuppressant, up to 25% of patients developed moderate or severe infusion reactions as described above.

Since then, hallmark studies have shown a relationship between ATI and infusion reactions. In a cohort of 125 consecutive patients with CD who were treated with episodic IFX infusions, IFX and ATI concentrations together with clinical data, side effects (including infusion reactions), and the use of concomitant medications before and 4, 8, and 12 weeks after each infusion were studied (Baert 2003). ATIs were detected in 61% of patients; almost all patients who developed ATI did so after the first or second infusion. The cumulative incidence of infusion reactions in this cohort of on demand treated patients was 27 percent. The vast majority of infusion reactions occurred during the second or third infusion. There was a strong correlation between the concentration of ATIs and the occurrence of infusion reactions. The median concentration of ATI was 20.1 µg/mL (95% confidence interval 3.0 to 22.6) at the time of a first infusion reaction, as compared with 3.2 µg/mL (95% confidence interval, 1.6 to 4.9) among patients without an infusion reaction ($p<0.001$). Concentrations of 8 µg/ml or higher predicted a higher risk of infusion reactions (RR 2.40; 95% CI 1.65 to 3.66; $p<0.001$).

A significant relation was also found between the serum IFX concentration measured 4 weeks after an infusion and the concentration of ATIs before that infusion ($r=0.34$, $p<0.001$). The median Infliximab concentration four weeks after an infusion was significantly lower among patients with an infusion reaction than among patients who never had a reaction (1.2 µg/ml vs. 14.1 µg/ml, $p<0.001$).

Once an infusion reaction occurred, the median duration of response to an infusion was shorter: 38.5 days (95% CI 34-51 days), as compared with 65 days (95% CI 56-71 days; $p<0.001$). Logistic regression analysis showed that the presence of ATIs was independently

associated with a shorter duration of response (p<0.001). Patients who were taking immunosuppressive agents had a lower incidence of antibodies (43%) than patients who were not taking immunosuppressive agents (75%) (p<0.01).

Another study observed similar findings (Farrell 2003). In a cohort of 53 patients an incidence of ATI of 36%, including all 7 patients with severe infusion reactions were found. The median ATI concentrations in these patients was 19.6 µg/ml. Eleven of 15 patients (73%) who lost response to Infliximab were ATI positive compared to none of 21 continuous responders. In addition to concurrent use of immunosuppressants, the administration of a second infusion within 8 weeks from the first was protective factors for ATI formation. In a second part of the study 80 patients were randomised to 200 mg of hydrocortisone or placebo before each infusion and found a lower incidence of ATI among steroid pre-treated subjects (26 vs. 42%). In a prospective study it was demonstrated that patients receiving immunosuppressants had lower ATI formation compared with patients receiving Infliximab alone (10% and 18%, respectively; p = 0.02) (Hanauer 2004).

Sequential measurement of ATI levels through the ACCENT 1 study has shown that ATIs may develop at any time during systematic or episodic retreatment (Hanauer 2004). However, ATI formation is more pronounced in patients treated episodically than in those treated in a scheduled manner, ranging around 30% after 72 weeks in the episodic strategy as compared to 10% and 7% in maintenance strategy with 5 mg/kg and 10 mg/kg, respectively. Important information provided by ACCENT 1 is that patients positive for ATI at any time point may later become negative and that globally, the proportion of patients positive for ATI at each time point is not increasing over time, even with episodic strategy. However maintenance therapy has proven superior to episodic treatment for various reasons, which are summarized in table 1. The most important advantages of maintenance therapy over episodic treatment include better response and remission rates, more thorough mucosal healing, and better

quality of life and reduced number of disease-related surgeries and hospitalizations. Recently the Sonic trial comparing Infliximab maintenance versus Infliximab plus azathioprine versus azathioprine monotherapy has shown remarkable and durable superiority for the combination therapy of Infliximab with immunosuppressant over an Infliximab maintenance regimen alone in immunosuppressive naïve patients (Colombel 2008).

Immunogenicity and Adalimumab

Allergic reactions

Adalimumab has been rarely reported to be related with systemic or injection site allergic reactions. These reactions can be drug- or host- specific and some of them seem to be IgE-mediated. In clinical trials with Adalimumab, approximately 1% of patients experienced allergic reactions such as allergic cutaneous eruptions, anaphylactic reaction, non-specified drug reaction and urticaria. In addition, anaphylaxis and angioneurotic edema have been reported rarely in post-marketing experience with Adalimumab.

Systemic allergic reactions clinically expressed as asthma have been also reported (Bennett 2005). In the CLASSIC-II trial the incidence of ANA formation was estimated at 19% (33/172 patients). All these 33 CD patients were also positive for anti-dsDNA. Of interest, 4/13 patients who were ANA-positive at baseline visit were ANA-negative at their final visit (Sandborn 2007). Adalimumab-induced lupus syndromes are rare (Martin 2008). In 1459 patients representing 1506 patient years only 3 lupus-like cases were recorded (Colombel 2007).

Clinical relevance of immunogenicity and Adalimumab

Adalimumab appears to be less immunogenic than IFX, confirming that in general chimeric antibodies are more immunogenic than human antibodies (Aarden 2008, Hwang 2005). The formation of human anti-human antibodies has been already reported long ago (Van de Putte 2004, FDA, Weinblatt 2003) however, it still remains unclear which part of Adalimumab induces anti-human antibody response (Bender 2007).

In the CLASSIC-I trial concomitant therapy with azathioprine and 6-mercaptopurine did not produce a significant change in serum concentrations of Adalimumab (Hanauer 2006). The CHARM (Colombel 2007) and the CLASSIC-II (Sandborn 2008) studies have shown ATA formation in 2.8% of CD patients and this did not differ between patients on or not on concomitant immunosuppressant. However, the CLASSIC II study is not powered nor designed to demonstrate the protective role of azathioprine, or methotrexate in the occurrence of ATAs. In addition, attempts to modulate the development of antibodies to anti-TNF therapies through concomitant immunosuppression do not necessarily prevent the need for dose escalation and/or reduced dosing interval.

In the CLASSIC II trial, three of the seven patients (43%) developing ATAs were in remission at week 24 and only two of seven (29%) were in remission at week 56 (Sandborn 2008). In addition, ATAs were associated with non-response to Adalimumab in a study with 30 CD patients previously exposed to Infliximab (West 2008). In this study of 30 CD patients receiving Adalimumab after Infliximab discontinuation, ATIs were positive in 57% of patients while ATAs were detected in 5/30 (17%) patients and 4 out of these five patients were Adalimumab non-responders. According to this study, patients previously treated with Infliximab with high levels of ATIs have a lower response rate to Adalimumab than patients with low levels of ATIs. The presence of ATAs was associated with low serum trough Adalimumab levels. The authors suggested that the reduced Adalimumab concentration was

because of the increased clearance of Adalimumab via the formation of immune complexes between ATAs and Adalimumab (West 2008).

Limits of ATI and ATA

Notably, not only are the techniques for measurement of antibodies different, even the results obtained by the different methods are not reported in a uniform or standardized manner that would enable reproducibility across studies. Thus, some report antibody levels in arbitrary units according to serial dilutions of a reference serum, whereas others reports measurements in microgram/ml. Moreover, there are hitherto no studies directly comparing the different methods outlined above, and thus it is hard to draw firm conclusions as to the most accurate and/or clinically beneficial method of detection. Such comparative studies are needed in order to ascertain the best methodology for antibody detection in terms of reproducibility, accuracy, and correlation with loss of clinical response to anti-TNF agents.

The real impact of auto-antibodies to Infliximab and Adalimumab in the mechanisms of the early and late allergic reactions and loss of response to these drugs deserves further studies before firm conclusions can be drawn.

The production of IgG/IgM/IgA antibodies directed against TNF mAbs induces a decreased efficacy. These antibodies could decrease the binding of mAbs to TNF through interaction of different parts of the mAbs: - Anti-VH/VL, more particularly anti-idiotopes antibodies directed against antigenic determinants related to the TNF binding site; - Anti-allotype antibodies leading to the formation of immune complexes.

Furthermore, the formation of these immune complexes (anti-TNF IgG1/anti-IgG immune complexes) probably accelerates the clearance of mAbs through capture by cells expressing

FcgRs. So far, little is known regarding the fine mapping of antibody specificity against anti-TNF mAbs.

5. IMMUNE AND NON-IMMUNE CLEARANCE OF ANTI-TNF MABS

Clearance of mAbs is a multi-factorial process, involving different mechanisms that are either antibody-dependent or host-dependent. The elimination of IgG is known to be concentration dependent, where half-life decreases as a function of increasing serum IgG concentrations. Catabolism is the dominant elimination mechanism of mAbs. However, the exact anatomical locations of this process have not been identified (Tabrizi 2006, Wang 2008). Specific binding sites on the Fc domain of the mAb that interact with the FcRn and the Fcγ receptors seem to play a crucial role. The impact of the Fab domain on clearance depends on the targeting antigen, namely if it is a soluble or a membrane-bound one.

The neonatal Fc receptor (FcRn): the salvage pathway

The neonatal Fc receptor (FcRn) is a major histocompatibility complex class-1-related receptor exerting a protective role regarding IgG catabolism. This specific intestinal transport receptor not only mediates neonatal IgG absorption, but also regulates IgG homeostasis (Brambell 1966). Mice genetically lacking expression of FcRn demonstrated rapid IgG elimination with a rate increased up to 10-15 fold, while no change was observed in the elimination of other immunoglobulins (Ghetie 1996 and 1997). Fab fragments that lack the Fc domain making them incapable for FcRn binding, demonstrate shorter half-lives than intact mAbs, although the presence of the PEG molecule also affects half-life. IgG binds FcRn via the Fc portion, remaining in this complex steady state as long as intracellular pH is mildly acidic and being released at physiologic pH (Raghavan 1995). Engineered mAbs should be delivered in very large doses in order to significantly alter serum IgG concentration, due to

the large quantity of endogenous IgG that is present in the body. On the other hand, they demonstrate altered (usually increased) affinity to human FcRns and thus altered (usually decreased) elimination rates especially through mutation of IgG Fc residues (Dall'Acqua 2002, Hinton 2006). Human FcRn selectively binds human IgG and this condition could explain the rapid clearance of murine IgGs from human circulation (Ober 2001). Human IgG 1, 2 and 4 exhibit longer elimination half-lives (~3 weeks) than IgG3 (one week) due to a higher affinity to FcRn.

Interaction of the mAbs with the target antigen (TNF): role of the variable region

Interaction with the target antigen can affect the elimination rate of mAbs. This condition is dose-dependent. Low mAb concentrations that do not saturate the antigen, demonstrate shorter half-life and subsequently a higher clearance rate compared to endogenous IgG; as the mAb's dose is increased and the antigen is progressively saturated, an increase in half-life and decrease in clearance rate is observed.

Monoclonal antibodies targeting soluble antigens usually interact with the FcRn and undergo a non specific clearance by the reticulo-endothelial system. Monoclonal antibodies interacting with membrane-associated internalizing antigens demonstrate a different elimination process characterized by internalization of the antibody-antigen complex, followed by degradation of the complex. In this case, the contribution of the antigen to mAb's clearance depends on antigen concentration and distribution as well as internalization and turnover rate (Tabrizi 2006, Wang 2008).

Role of Fcγ receptors in the clearance of anti-TNF mAbs

FcγRs belong to the immunoglobulin superfamily and induce phagocytosis and destruction of opsonized microbes via complement dependent or antibody dependent cell–mediated cytotoxicity. This family includes several different isoforms, namely FcγRI (CD64), FcγRIIA

(CD32), FcγRIIB (CD32), FcγRIIIA (CD16a) and FcγRIIIB (CD16b), which differ in their antibody affinities due to their different molecular structure. FcγRI demonstrates the highest degree of affinity with the IgG and FcγRIIB the lowest (Tabrizi 2006, Wang 2008). On the other hand, different IgG isotypes such as IgG1, 2, 3 and 4, demonstrate unique recognition and activation profiles, when interacting with various FcγRs (Indik 1995). The above mentioned characteristics regarding interaction between different FcγRs with different IgG isotypes could also affect pharmacokinetics and clearance of the IgG mAbs from the cells of the reticulo-endothelial system. For example, homozygous FcγRIIIA-F/F158 polymorphism led to more rapid elimination of opsonized red blood cells coated with an anti-D IgG3 mAb by phagocytic cells in humans (Kumpel 2003).

Immune complexes containing mAbs can be eliminated through interactions with FcγRs. Different couples of immune complexes can be formed, made of TNF and mAbs, or of mAbs and anti-mAbs (ATI or ATA). The clearance efficacy is likely related to the FcγRII and FcγRIII polymorphisms, hence leading to various clinical consequences depending on the patient.

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STATEMENTS

TABLE 1: PRIMARY NON RESPONSE

Key messages

1. Approximately a 1/3 of patients do not show response and 2/3 do not achieve remission.

2. When selecting patients with active CD (assessed by inflammatory markers and/or lesion assessment), the absence of response is rare and ranges between 10 and 30%, while it is around 40% in UC.

3. Maximal response rate is reached after 12 weeks

4. A broad range of "response intensity" exists and full response characterized by clinical remission and tissue healing only occurs in a minority of patients (around 30%).

5. Response rate may be influenced by disease location, disease duration, active inflammation, strictures, disease type, anti-TNF dose, co-treatment

Questions to be addressed in the future

1. What is the best definition of non response (criteria, timing)?

2. What is the optimal induction regimen (dose, number and frequency of dosage)?

3. What is the real benefit of co-treatments?

4. What are the response rates when treating stricturing CD?

5. What are the response rates in refractory proctitis?

TABLE 2: SECONDARY NON RESPONSE

Key messages

1. Loss of response varies from around 50% per year in placebo-controlled trials to a slightly more than 10% per year in smaller studies and monocentric experiences in which treatment optimization (including dose escalation and dose interval changes) is allowed.

2. Factors that may influence loss of response include steroid and immunosuppressive co-treatments

3. Treatment optimization with increased dose or shortened interval allowed recovering response in 50-90% of the patients

4. The optimal method for dose optimization is yet to be determined

Questions to be addressed in the future

1. What is the best definition of loss of response?

2. What is the impact of induction regimen on long term response and risk of loss of response?

3. What are the best optimization regimens (dose increase, interval shortening, re-induction or co-treatment)?

TABLE 3: PHARMACOKINETICS

Key messages

1. Elimination of monoclonal antibodies varies between individuals and is most likely influenced by immunogenicity (anti drug antibodies) and by differential clearance.

2. The therapeutic window concept postulates that a threshold trough concentration is required for therapeutic efficacy.

3. The pharmacokinetics of monoclonal antibodies is determined by three basic factors: the mode of administration, drug half lives and peak-through concentrations in serum

4. The serum level of the monoclonal antibody is significantly affected by antibody formation

5. Loss of response to anti-TNF agents is only partly explained by antibody formation and immunogenicity; other factors including individual differences in drug clearance are likely to play a role as well

Questions to be addressed in the future

1. What is the correlation between concentrations of the anti-TNF agent in the serum and in the inflamed tissue?

2. Are factors than immunogenicity influencing levels of anti-TNF in the blood?

3. Can the interplay between monoclonal antibodies and antigens (i.e. antigen saturation and distribution) affect IgG metabolism?

TABLE 4: IN CASE OF LOSS OF RESPONSE, DRUG TROUGH LEVELS AND ANTIBODY MEASUREMENTS COULD AID IN DECISION MAKING

1. In patients with undetectable drug levels, antibody measurement may be useful. Most will likely have high anti-drug antibody titers and switching the drug is probably the best option**.**

2. In patients with low to intermediate drug readouts, an attempt to restore trough levels by dose escalation or shortening infusion/injection intervals should be considered.

3. In patients with symptoms suggestive of active disease despite high trough levels, disease reassessment including the use of CRP, fecal calprotectin, and/or imaging should be performed.

4. If these patients have active inflammation and no infection, use of a compound with another mechanism of action should be considered.

TABLE 5: IMMUNE AND NON IMMUNE CLEARANCE

Key messages

1. Anti-drug antibodies can lead to loss of response by increasing drug clearance

2. Anti-drug antibodies are probably under-detected due to technical shortcomings and imperfect test timing

3. Monoclonal antibody humanization reduces antigenicity, but is inferior to homology. Human antibodies may be also immunogenic.

4. "Neutralizing" anti-idiotypic antibodies could lead to a complete or partial inhibition of the anti-TNF mAbs binding to TNF

5. Scarce data exist on the role of co-existing PEG molecules in clearance of biologic agents consisting of Fab fragments

6. Applying targeting mutations in certain positions on the Fc domain could influence the interplay between the monoclonal antibody and FcRn or Fcγ receptors

Questions to be addressed in the future

1. What causes formation of antibodies to anti-TNF monoclonal antibodies in some patients, but not in others?

2. How could we explain the differences between patients with high and low concentrations of anti-drug antibodies?

3. What is the relative role of anti-drug antibodies on loss of response?

4. What is the preferred technique to measure anti-drug antibodies?

5. How to prevent anti-drug antibodies formation? What is the risk/benefit ratio of concomitant treatments?

6. How should anti-drug antibodies presence direct our management?

7. Can optimization of the pharmacokinetic properties of monoclonal antibodies produce more efficient molecules regarding metabolism?