DISCOVERY MEDICINE®
www.discoverymedicine.com
ISSN: 1539-6509; eISSN: 1944-7930

Personalized Medicine: Theranostics (Therapeutics Diagnostics) Essential for Rational Use of Tumor Necrosis Factor-alpha Antagonists

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Abstract: With the discovery of the central pathogenic role of tumor necrosis factor (TNF)-α in many immunoinflammatory diseases, specific inhibition of this pleiotropic cytokine has revolutionized the treatment of patients with several non-infectious inflammatory disorders. As a result, genetically engineered anti-TNF-α antibody constructs now constitute one of the heaviest medicinal expenditures in many countries. All currently used TNF antagonists may dramatically lower disease activity and, in some patients, induce remission. Unfortunately, however, not all patients respond favorably, and safety can be severely impaired by immunogenicity, i.e., the ability of a drug to induce anti-drug antibodies (ADA). Assessment of ADA is therefore an important component of the evaluation of drug safety in both pre-clinical and clinical studies and in the process of developing less immunogenic and safer biopharmaceuticals. Therapeutics diagnostics, also called theranostics, i.e., monitoring functional drug levels and neutralizing ADA in the circulation, is central to more effective use of biopharmaceuticals. Hence, testing-based strategies rather than empirical dose-escalation may provide more cost-effective use of TNF antagonists as this allows therapies tailored according to individual requirements rather than the current universal approach to diagnosis. The objective of the present review is to discuss the reasons for recommending theranostics to implement an individualized use of TNF antagonists and to highlight some of the methodological obstacles that have obscured cost-effective ways of using these therapies. [*Discovery Medicine* 15(83):201-211, April 2013]

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Introduction

The last decade has seen a revolution in the treatment of patients with various immunoinflammatory diseases, for example arthritic diseases [e.g., rheumatoid arthritis (RA) and ankylosing spondylitis], inflammatory bowel diseases [e.g., Crohn's disease (CD) and ulcerative colitis], skin diseases (e.g., psoriasis and hidradenitis suppurativa), non-infectious diseases of the eye (e.g., diabetic macular edema and age-related macular degeneration), and refractory asthma (Langford, 2008; Lin *et al*., 2008; Emery *et al*., 2009; Allez *et al*., 2010; Desai and Brightling, 2010; Furst *et al*., 2011; Jemec, 2013). Thus, antibody constructs that specifically antagonize the inflammatory cytokine tumor necrosis factor (TNF)-α have dramatically improved outcomes for patients refractory or intolerant to conventional treatments, reducing corticosteroid requirements and, in some cases, inducing long-term remission (Tanaka, 2012).

The anti-TNF-biopharmaceuticals for systemic administration are the human TNF receptor/IgG1 fusion protein, etanercept/Enbrel; the chimeric human/mouse mAb, infliximab/Remicade; the human mAbs, adalimumab/Humira and golimumab/Simponi; and the mAb/Fab fragment, certolizumab pegol/Cimzia. They all target both soluble and membrane-associated forms of TNF-α, thus inhibiting TNF-α from triggering cellular TNF-receptors. The effects have been so dramatic, and the number of patients who benefit so large that TNF-α-targeting drugs now constitute one of the heaviest medicinal expenditures in many countries.

The massive use of TNF antagonists has confronted clinicians with several challenges. Apart from immunosuppression and other side-effects, up to 50% of patients sooner or later experience treatment failure (Cassinotti and Travis, 2009; Afif *et al*., 2010; Aikawa *et al*., 2010; Allez *et al*., 2010; Krieckaert *et al*., 2010; Bartelds *et al*., 2011; Ben-Horin and Chowers, 2011;

Danese *et al*., 2011; Yanai and Hanauer, 2011; Atzeni *et al*., 2012; Chaparro *et al*., 2012; Colombel *et al*., 2012; Garces *et al*., 2012). As there is currently no established standard for handling patients with insufficient effect of TNF antagonists, clinicians are left with only a few choices when the drugs fail -- all based on clinical outcome. They can wait and see, empirically intensify treatment with the existing drug, switch to another TNF antagonist, or switch to an entirely different class of drugs. This 'trial-and-error' approach has several disadvantages. Patients with inflammation in the presence of otherwise therapeutic drug levels are not identified, suffering continues if the new and empirically chosen drug is also ineffective, and there is a risk of irreversible tissue damage while physicians search for an effective new drug. It is also important to realize the enormous financial consequences of unsuccessful 'trials.'

One approach to deal with these problems is to use therapeutics diagnostics, or theranostics, so that clinicians can identify patients for whom a medication, or a change in medication is likely to work (Palekar-Shanbhag *et al*., 2012). It offers the possibility of tailoring therapy according to individual needs as opposed to the standard generic approach to diagnosis, and it aims at reducing delays in effective treatment. Testings for anti-drug antibodies (ADA) may also help assessing the risk of potentially dangerous reactions caused by drug immunogenicity. Finally, theranostics has the potential to improve the cost-effectiveness of already expensive therapies.

Clinical Use of TNF Antagonists

Apart from infliximab, which is given intravenously, all systemically used biological TNF antagonists are formulated for self-administration through subcutaneous injections. Because of the high costs associated with prolonged therapies, episodic treatment with discontinuation of the drugs during clinical remission is used in some patients even though this strategy is associated with insufficient responses and adverse reactions (Steenholdt *et al*., 2011a).

Inadequate effect of TNF antagonists

Most patients respond favorably to TNF antagonists, at least initially, but some do not. It is for example unknown why certain TNF antagonists are effective in some but not in other chronic inflammatory disorders (Assasi *et al*., 2010). In general, patients with inadequate effects either do not respond at all (primary response failure) or they respond initially but have later relapses (secondary response failure) despite increased dosage and/or more frequent administration of the drugs (Strand *et al*., 2007; Bendtzen *et al*., 2009; Allez *et al*., 2010; Krieckaert *et al*., 2010; Furst *et al*., 2011). The mechanisms underlying these failures are not entirely clear, partly because the problem has received little attention. Proposed explanations for drug failure include problems related to bioavailability, pharmacokinetics (PK), and pharmacodynamics (PD) in addtion to immunogenicity with development of ADA (Ainsworth *et al*., 2008; Wijbrandts *et al*., 2008; Bendtzen, 2012).

PK, PD, and primary response failure

It is likely that at least some of the above mentioned problems could be overcome if therapists had knowledge of the PK and PD of the drugs in individual patients. For example, drug levels in sera sampled at the end of a therapeutic cycle (trough) are surrogate PK markers, and these levels have been shown to vary considerably between patients on standardized dose regimens with low levels correlating with (later) loss of response (Bendtzen *et al*., 2006; Ternant *et al*., 2008). These variations relate to structure and formulation of the drugs, and route and frequency of administration, but they also seem to depend on patient-related features such as age, sex, weight, intercurrent diseases, concomitant medications, and the ability of an individual to mount an immune response.

If primary nonresponders are routinely monitored for circulating levels of the TNF antagonist or, better yet, for levels of TNF-α-neutralizing activity, one would be able to adjust treatment on the basis of patient-relevant evidence which is likely to be much more cost-effective. For example, in the absence of ADA it would be logical to increase the dosage of a drug, or to shorten the dosing interval in patients with insufficient TNF-α-neutralizing capacity. On the other hand, some patients with primary response failure have therapeutic or even supra-therapeutic drug levels (Ainsworth *et al*., 2008). These patients are not likely to benefit from intensified therapy, and because the patients are unresponsive despite already high blood levels of anti-TNF-α activity, switching to another TNF antagonist is also likely to be ineffective. Indeed, the presence of supra-therapeutic concentrations of bioactive drug in unresponsive patients raises the question whether TNF-α is of key pathogenetic importance in all cases diagnosed as a single disease entity (Ainsworth *et al*., 2008; Wijbrandts *et al*., 2008). If not, RA, CD, psoriasis, and many other diseases, where the diagnoses rest primarily or solely on clinical criteria, may constitute pathophysiologically heterogeneous groups of diseases, some of which responding to TNF antagonists, others not (Bendtzen, 2012). Nonetheless, monitoring functionally active

drugs in the circulation is warranted in patients with primary response failure because demonstration of high drug levels may save months of futile and expensive therapy and allow earlier shift to effective treatment (Bendtzen, 2011).

Immunogenicity and secondary response failure

Many patients who meet the criteria for an initial clinical response sooner or later lose effect of TNF antagonists, and the one-year drug survival rates may in some cases decline to less than 20% mainly due to loss of efficacy (Allez *et al*., 2010; Furst *et al*., 2011; Brunasso *et al*., 2012; Emery, 2012). Secondary response failure can in some patients be related to individual differences in drug bioavailability and PK, as this may lead to inadequate drug levels in the circulation and in affected tissues. PD issues involving the mechanisms underlying inflammation in the affected tissues, may also be involved. Infections and alterations in concomitant therapies, or the introduction of new ones, may for example affect pathophysiological processes in tissues also affected by the original disease. The major contributor to secondary response failure, however, appears to be immunogenicity leading to production of ADA with removal of the drug from the circulation and/or direct neutralization of drug activity.

Anti-drug antibodies (ADA)

Repeated injections of biopharmaceuticals may trigger production of ADA (Kromminga and Schellekens, 2005; Shankar *et al*., 2006; Strand *et al*., 2007). This is hardly surprising because administration of many protein drugs resemble vaccination procedures: repetitive subcutaneous injections of non-self proteins. All currently used anti-TNF biopharmaceuticals are *non-self* glycoproteins, including the so-called fully human antibodies (adalimumab and golimumab) that contain TNF-binding idiotopes that are not part of a normal human antibody repertoire (Bendtzen, 2012). It is therefore to be expected that TNF antagonists also induce ADA (Fefferman and Farrell, 2005; Bendtzen *et al*., 2006; Wolbink *et al*., 2006; Aarden *et al*., 2008; Ebert *et al*., 2008; West *et al*., 2008; Bendtzen *et al*., 2009; Cassinotti and Travis, 2009; Karmiris *et al*., 2009; Petitpain *et al*., 2009; Wolbink *et al*., 2009; Aikawa *et al*., 2010; Allez *et al*., 2010; Jamnitski *et al*., 2010; Krieckaert *et al*., 2010; Lecluse *et al*., 2010; Makol *et al*., 2010; Bartelds *et al*., 2011; Bendtzen, 2011; Furst *et al*., 2011; Korswagen *et al*., 2011; Steenholdt *et al*., 2011a; Yanai and Hanauer, 2011; Emery, 2012). What is surprising is that it has taken more than a decade to realize this important problem. In RA and CD patients, for example, half the patients with initial response to infliximab experience flare of disease after months of therapy, and several studies have now shown that ADA are closely associated with these events. This frequency is even likely to be a low estimate, because the full impact of drug immunogenicity is realized only if patients are monitored for ADA on a routine basis or, at the very least, every time treatment failure or side-effect occur.

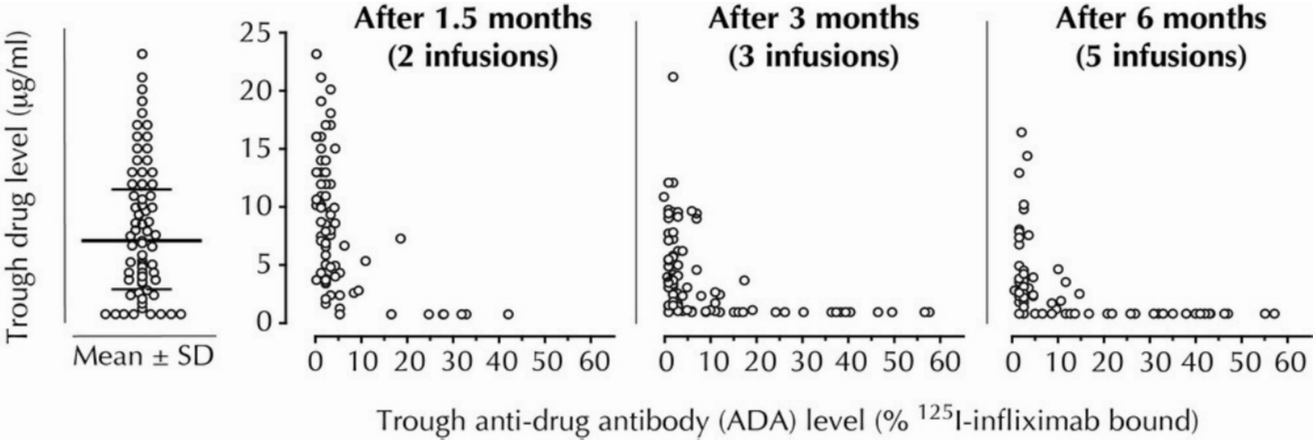

Figure 1. Interindividual differences in serum levels of infliximab, and dynamic influence of ADA on drug PK. Serum levels of infliximab (Y-axes) and anti-infliximab ADA were measured in 106 RA patients using RIA. Trough level testings were carried out on sera drawn immediately before the 3rd intravenous infusion of infliximab 1.5 months after start of therapy, and before the 4th and 6th infusions, respectively (right panels). The drug was administered at the recommended dosage of 3 mg/kg. The left panel is a scatter plot showing mean value ± standard deviations (SD) of the drug levels achieved after the 2nd infusion. Note the gradual disappearance of the drug as soon as ADA develop. Approximately half the patients (44%) were ADA-positive after 6 months of therapy. Extended with permission from *Arthritis and Rheumatism* (Bendtzen *et al*., 2006).

If not, as is the usual approach today, clinicians will never know that ADA could be the underlying cause of therapeutic failure, and side-effects.

The latter is highlighted by reports on potentially serious side-effects that seem to be mediated by drug-ADA immune complexes, for example, infusion reactions, serum sickness, bronchospasm, and Arthus reactions (Wolbink *et al*., 2005; Bendtzen *et al*., 2006; Wolbink *et al*., 2006; Descotes and Gouraud, 2008; Dubey *et al*., 2009; Bendtzen, 2011; Korswagen *et al*., 2011). The problem of drug immunogenicity becomes even more important as evidence from infliximab-treated CD patients show highly variable kinetics of ADA in the circulation (Steenholdt *et al*., 2012). In some patients, ADA persist for years even after discontinuation of therapy, whereas others clear ADA from the circulation during continued therapy. In view of the above findings, it is remarkable that the vast majority of patients still receive TNF antagonists without consideration of the fact that development of ADA poses a safety risk (Cheifetz and Mayer, 2005; Hwang and Foote, 2005; Tangri *et al*., 2005; De Groot and Scott, 2007; Bendtzen, 2011; 2012).

Investigations have shown that drug bioactivity disappears from the circulation as soon as ADA appears (Bendtzen *et al*., 2006; van den Bemt *et al*., 2011) (Figure 1). Not surprisingly, several other investigations of RA and CD patients have shown that low blood levels of infliximab, adalimumab, and etanercept, and the presence of ADA correlate with the requirement for dose increase, therapeutic failure, and infusion reactions (St. Clair *et al*., 2002; Wolbink *et al*., 2005; Mulleman *et al*., 2009; Radstake *et al*., 2009; Afif *et al*., 2010; Bartelds *et al*., 2011; Jamnitski *et al*., 2011; Steenholdt *et al*., 2011a; Yanai and Hanauer, 2011).

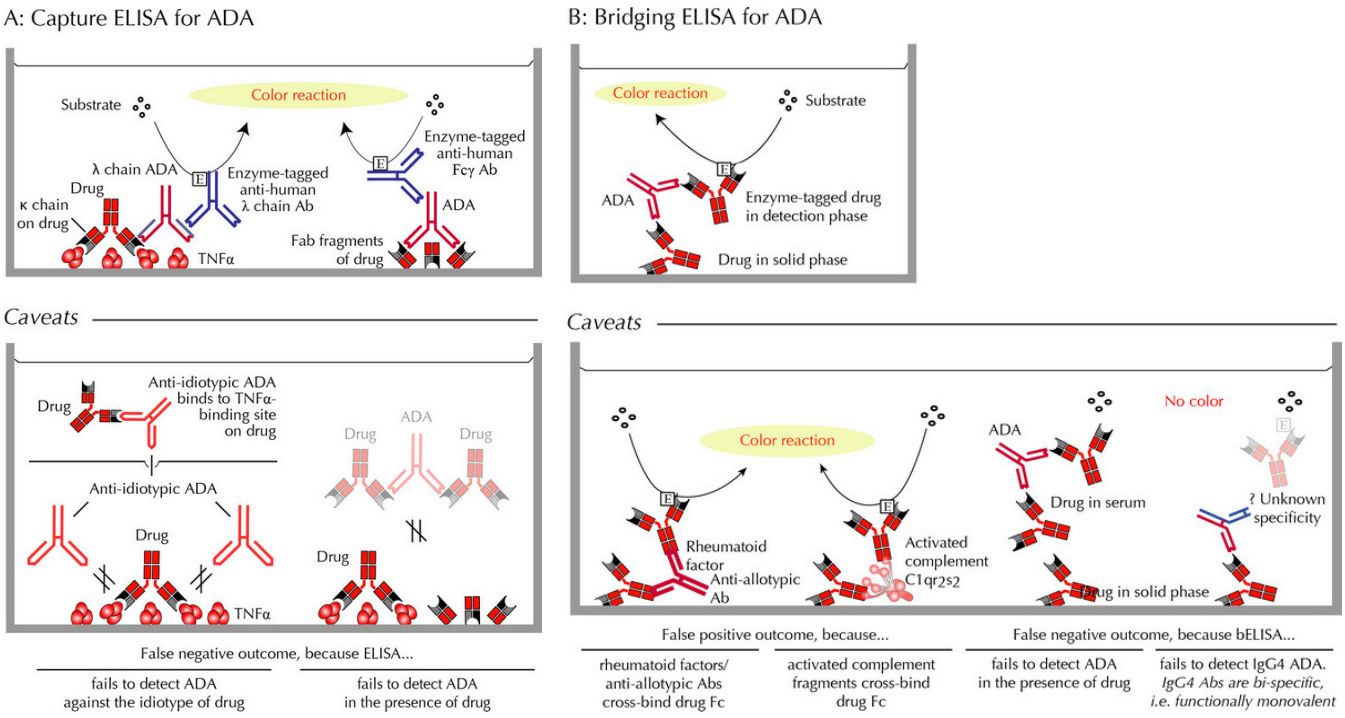

Figure 2. ELISAs for ADA. **A**: Capture ELISA. Left upper panel: λ light-chain ADA, bound to the TNF antagonist captured on TNF-α-coated plastic wells, are detected by enzyme-labeled anti-human λ light-chain antibody (Ab). Right upper panel: Alternatively, ADA captured on plastic-adherent Fab fragments of the drug, are detected by enzyme-labeled anti-human Fcγ Ab. Left lower panel: False negative ADA testings may arise from failure to detect anti-idiotypic ADA. Right figures in the lower panel: False negative ADA testings due to high drug-sensitivity, i.e., inability to detect ADA in the presence of drug. **B**: Bridging ELISA. Upper panel: Bridging ELISA depends on the bivalency of primarily IgG Ab (and multivalency of IgA and IgM Ab) and, hence, the ability of these immunoglobulin classes to 'bridge' a drug molecule preabsorbed to a plastic plate with an added enzyme-labeled drug molecule. Left two figures in the lower panels: False positive ADA testings may arise from cross-binding of drug Fc-fragments by sera containing rheumatoid factor and/or anti-allotypic Abs, or activated complement components in sera of patients with high inflammatory activity. Right two figures in the lower panels: Most often, however, bridging ELISA generates false negative results for ADA because they are highly drug-sensitive and/or because of failure to detect IgG4 ADA which predominate after prolonged immunizations.

Assays for TNF Antagonists and ADA

An important and often overlooked aspect of using testing-based strategies is the ability of assays to accurately and reliably measure levels of functionally active drug and ADA (Bendtzen, 2012). Detections of ADA, for example, are generally hampered by the fact that most TNF antagonists are by themselves immunoglobulins, and by the complexity of measuring antibodies against antibodies in non-functional binding assays.

Immunoassays for ADA binding

Enzyme-linked immunosorbent assays (ELISAs) are the most commonly used tests for ADA in patient serum. Unfortunately, however, all solid-phase assays have several notable limitations for clinical use (Bendtzen, 2012) (Figure 2). These include difficulties for certain capture ELISAs to detect anti-idiotypic antibodies because idiotopes residing in the TNF-α binding site(s) of an antibody drug are masked by TNF-α in the capture phase (Figure 2A). The frequently used bridging ELISA modification also fails to detect IgG4 antibodies, a major isotype of ADA after prolonged immunizations (Svenson *et al*., 2007; Bendtzen, 2012; van Schouwenburg *et al*., 2012). This is because IgG4 antibodies are functionally monovalent and therefore cannot 'bridge' in this type of binding assay (Figure 2B). Perhaps more importantly, ELISA is highly sensitive to the presence of drug in the sample being tested for ADA, giving rise to false negative findings (Hart *et al*., 2011; Bendtzen, 2012). Some investigators consequently report ADA status as inconclusive in sera tested with ELISA if drug is detectable (Yanai and Hanauer, 2011). This has been estimated to be the case in about half the patients in clinical trials.

ELISAs and other solid-phase assays are also known to report false positive findings for example from neoepitope formation and from nonspecific binding of low-affinity antibodies, including heterophilic antibodies, and also from rheumatoid factors and/or activated complement which may cross-bind IgG Fc fragments in bridging ELISA (Figure 2B).

As tools for therapeutic guidance, it is essential to realize that binding assays do not discriminate between neutralizing and non-neutralizing ADA, an issue recognized in 2007 by the European regulatory authorities (European Medicines Agency, 2007). With this in mind and the limited *in vivo* relevance of solid-phase techniques in general, the wide use of ELISA in recent years may have contributed to the confusion surrounding drug immunogenicity -- and will continue to do so as long as therapeutic relevance is ignored when assessing ADA in the clinical setting. Newer solid-phase binding assays, including those based on bead- and chip technologies, may have similar disadvantages as they report antibody binding to drugs in a non-homogeneous and, possibly, aggregated or otherwise denatured form (Bendtzen, 2012).

Semi fluid-phase enzyme-immunoassay (EIA). In this 'reverse' EIA, patient serum and biotinylated drug are allowed to preincubate in solute phase before being developed on avidin-coated plates (Bendtzen and Svenson, 2011). In a newer modification, serum is first added to plastic wells precoated with protein G which selectively binds to the Fc parts of IgG molecules irrespective of their antigen specificities (Figure 3). After removal of all non-IgG serum components (by washing), any immobilized IgG ADA are visualized by addition of the enzyme-tagged drug. This modification also allows ADA to bind to drug that has not been denatured by prior plastic absorption as in an ELISA setup.

Fluid-phase radioimmunoassay (RIA) for ADA has generated promising results in patients with RA and CD (Bendtzen *et al*., 2006; Bartelds *et al*., 2011; Bendtzen and Svenson, 2011; Steenholdt *et al*., 2011b; Krieckaert *et al*., 2012). Most of these assays are less sensitive to artifacts encountered in solid-phase assays and therefore thought to better reflect the *in vivo* conditions (Figure 4). In contrast to ELISA, RIA is highly sensitive and measures ADA also in the presence of

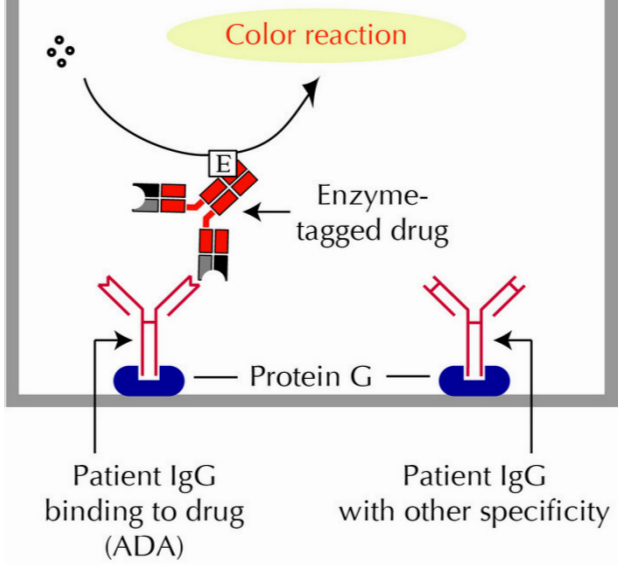

Figure 3. EIA for ADA.

limited amounts of drug. In addition, RIA detects all isotypes and subclasses of immunoglobulins that bind to the drug, including IgG4. The major limitation of RIA is the need for advanced laboratory facilities and the fact that these assays also fail to detect whether the reported ADA are neutralizing or not.

Homogeneous mobility-shift assay (HMSA) uses size exclusion high-performance liquid chromatography to determine concentrations of ADA (Figure 5). This technology was recently introduced in North America as a replacement for ELISAs for ADA detection (Wang *et al*., 2012). The clinical potential of HMSA is under investigation, but its rather expensive setup may limit routine use. The fact that immune complexes may split during chromatography giving rise to drug detection in the presence of ADA is a potential problem. As with other binding assays, HMSA does not inform on the functionality of the reported ADA.

Cell-based assay for neutralizing ADA

Reporter-gene assay (RGA) is the most recent development (Lallemand *et al*., 2011; Bendtzen, 2012) (Figure 6). In contrast to binding assays, RGA measures the functionality of both drugs and ADA, i.e., the TNF-inhibitory effect of drugs and neutralizing ADA, respectively. Though at present largely unexplored in clinical trials, it may be desirable to use RGA for patient monitoring as it reports ADA that bind with sufficient avidity and to a locality on the drug that interferes with drug activity at the cellular TNF-receptor level. This assay therefore seems to resemble the *in vivo* conditions under which TNF antagonists are believed to function.

The usual limitations of cell-based assays have been overcome by the development of an RGA that allows both drug activity and neutralizing ADA to be quantified independent of serum matrix effects and cell-numbers. In addition, the use of assay-ready cells stored at -80°C for extended periods without loss of sensitivity obviates the need for continuous cell cultivation. The assay reports ADA within a few hours, and it is easily modified for quantification of residual drug activity and neutralizing ADA in patients treated with all known anti-TNF biopharmaceuticals.

Testing-based Strategies as Clinical Utility

Some investigators hold the view that there is little need to monitor drug levels, let alone ADA levels, if patients are doing well on standard regimens. Some even feel that ADA are of limited importance because there are not always observable consequences of ADA development. These assumptions are fostered by the use of assays that have little clinical relevance, and even on assays known to yield false results in the clinical setting (Aarden *et al*., 2008; Bendtzen, 2012).

The wide use of bridging ELISA for detecting ADA in patient serum is perhaps the most problematic example, as this type of assay as mentioned previously cannot detect ADA in the presence of drug (Hart *et al*., 2011). Consequently, the extensive use of bridging ELISA and other assays that underestimate ADA in the circulation and/or do not reflect the *in vivo* conditions in patients may have contributed considerably to the confusion many investigators feel regarding the therapeutic importance of drug immunogenicity.

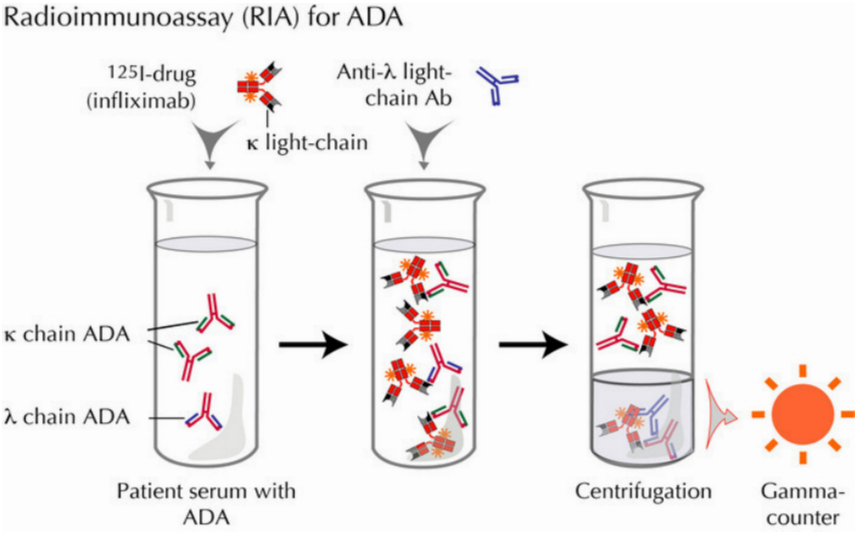

Figure 4. RIA for ADA. This RIA detects all immunoglobulin isotypes of ADA against all currently registered TNF antagonists. Free and immunoglobulin-bound tracer are separated by spinning down only the radiolabeled drug binding to λ light-chain ADA in complex with anti-λ light-chain Ab.

Some clinicians also find it sufficient to monitor drug levels alone (without ADA). The rationale behind this is that ADA should reflect itself in sub-therapeutic or undetectable drug levels in the circulation. This is unfortunate for several reasons:

1. There are other causes of low drug levels than ADA, for example improper handling/storage (causing drug aggregation), compliance problems (most TNF antagonists

are administered by patients themselves), and serum sampling after prolonged injection intervals (resulting in lower than 'normal' drug levels). Non-ADA induced degradation and/or elimination of antibody constructs may also play a role (Ordas *et al*., 2012).

2. If therapists continue to immunize a patient with undiscovered ADA, possibly even with increased doses because of low drug levels, extended therapy becomes both costly and ineffective (van der Maas *et al*., 2012). It also compromises safety because the risk of adverse events increases in the presence of ADA.

3. If low drug levels are being determined by ELISA or other binding assays, these data do not reveal the function of the drug, i.e., the TNF-α neutralizing capacity. Several serum factors are known to interfere with ELISA, including normally occurring serum components such as C-reactive protein and albumin, and non-neutralizing antibodies (Ordas *et al*., 2012).

Assuming the use of accurate and sensitive assays that reflect the *in vivo* conditions, how should theranostics be implemented in order to optimize efficacy and safety in individual patients? In case of an insufficient response to a TNF antagonist, one should initially confirm that symptoms originate from increased activity of the underlying disease. Non-inflammatory symptoms should be realized, as should inflammatory activity due to reasons other than relapse of the disease being treated (infections, ischemia, etc.). In case of assumed drug failure, it is suggested to measure serum levels of both drug and ADA to better understand the pharmacoimmunological realities in each individual patient. A previously proposed algorithm considers four principal situations (Bendtzen *et al*., 2009) (Figure 7):

1. A PD issue where loss of response occurs in the presence of moderate or even high TNF-α-neutralizing serum capacity. This is speculated to arise from activation of alternative immunoinflammatory pathways bypassing TNF-α as a central pathogenic factor. Many patients with primary response failure appear to belong to this subgroup, and these patients are not likely to benefit from dose escalation -- or change to another TNF antagonist. They should be switched directly to another therapeutic principle.

2. Optimal levels of drug in the presence of ADA may arise from false positive ELISA testings, or they may be due to detection of low-avidity and/or otherwise functionally inactive ADA. In such cases sera should be retested with a cell-based assay both for functionally active drug and drug-neutralizing ADA. In case of unchanged findings, patients are considered suffering from a PD issue, and they should be treated as those in group 1.

3. Suboptimal levels of drug despite assured compliance, and no demonstration of neutralizing ADA. This condition is considered due to non-antibody-mediated inadequate bioavailability and/or PK issues with increased drug turnover for example due to increased 'inflammatory load' with elevated expression of TNF-α in the affected tissues and/or increased drug clearance. As there is only limited information regarding factors other than ADA that influence the turnover of TNF antagonists, it is suggested that patients in this group receive intensified therapy with the already administered TNF antagonist.

4. Immunogenicity with generation of drug-neutralizing ADA resulting in insufficient TNF-α blockade and/or increased drug clearance. Because ADA are usually drug specific, patients in this category should benefit from switching to a different TNF antagonist.

The above algorithm has been supported by other investigators (Afif *et al*., 2010; Yanai and Hanauer, 2011; Ordas *et al*., 2012) and has recently been tested in a prospective, randomized, controlled, and blinded trial involving CD patients treated with infliximab (Steenholdt *et al*., 2013). Compared to routine escalation of drug dosage, therapy guided by the algorithm reduced the average treatment costs per patient by 30% to 50% without compromising clinical efficacy.

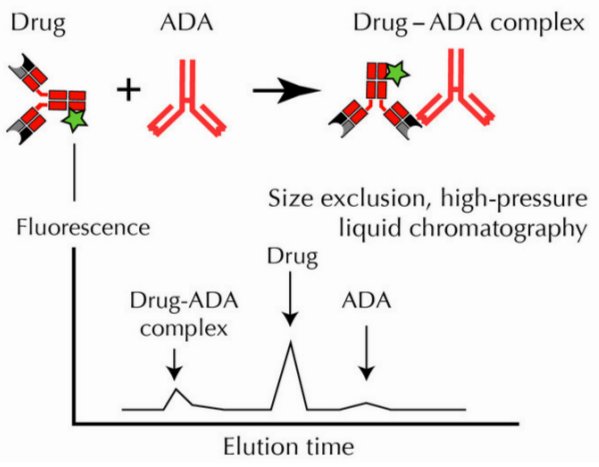

Figure 5. HMSA for ADA. HMSA detects all immunoglobulin isotypes of ADA by size-exclusion high-performance liquid chromatography and subsequent quantification of fluorescence-labeled drug in free form and in complex with ADA (Wang *et al*., 2012).

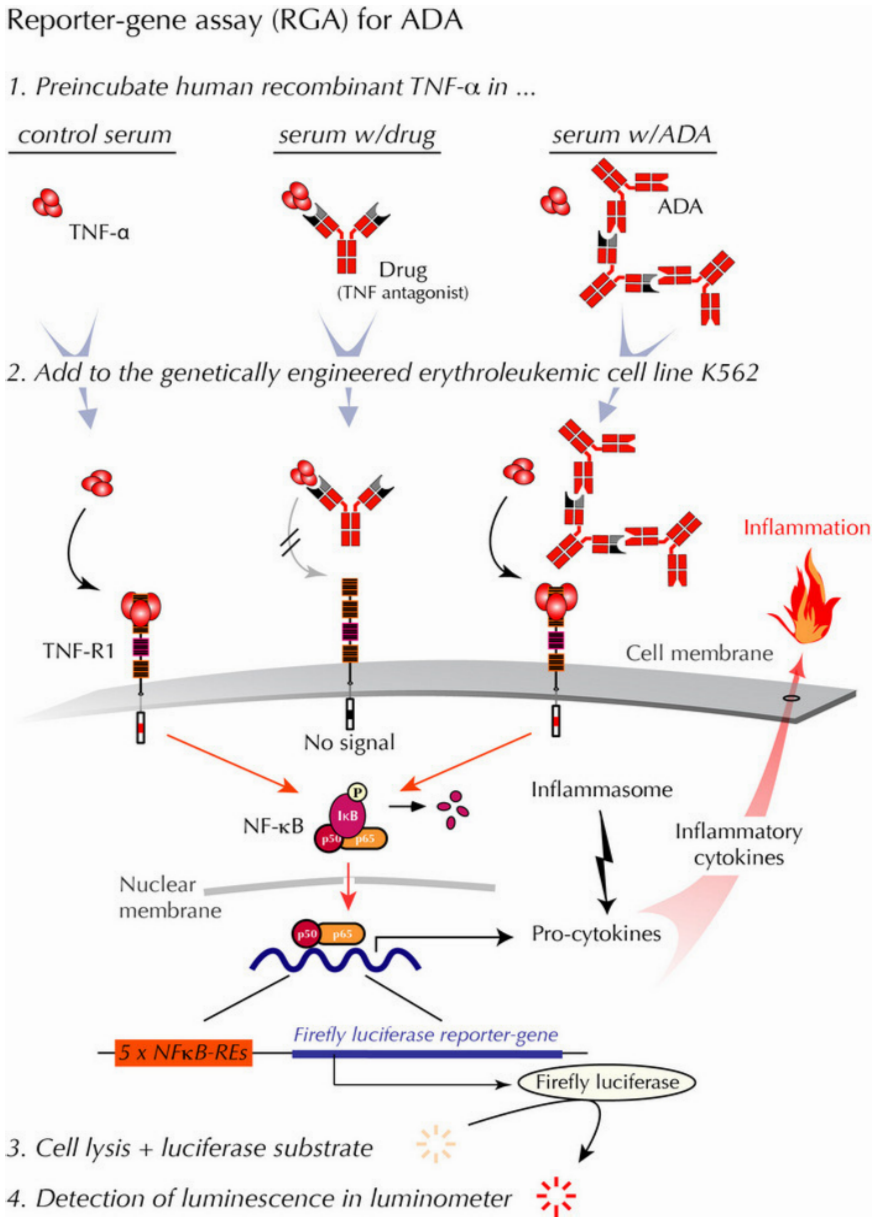

Figure 6. RGA for neutralizing ADA (and functional drug levels). This cell-based assay reports functional levels of drug-neutralizing ADA and, in addition, TNF-α-neutralizing activity of all currently used anti-TNF-α drugs (Lallemand *et al*., 2011). Steps 1 to 4 of one assay are indicated in italic. When human recombinant TNF-α in normal serum is added to the cells (left upper part), the cytokine initiates intracellular signalling through the surface TNF-receptor, type 1 (TNF-R1), thus activating the cytoplasmic nuclear factor (NF)-κB. The active components of this transcription factor are then transported to the nucleus where they bind to NF-κB response elements (NF-κB-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 is quantified in a luminometer. When TNF-α is preincubated with patient serum containing a TNF antagonist and then added to the cells (middle upper panel), the drug, if functional, neutralizes the effect of TNF-α, and no cell-signal is initiated. When TNF-α is preincubated with patient serum containing drug-neutralizing ADA and then added to the cells (right upper panel), the drug no longer interferes with TNF-α-mediated signalling resulting in a luminescence output.

Conclusions

1. Therapies with anti-TNF-α antibody constructs are often effective in patients suffering from a host of chronic immunoinflammatory diseases.

2. Unfortunately, about one third of patients with RA and CD do not respond to TNF antagonists, and one third lose effect over time despite ongoing therapy.

3. PK issues correlate with inadequate responses to TNF antagonists. This results in insufficient drug levels to adequately neutralize TNF-α in the circulation and in target tissues. Drug immunogenicity is the underlying factor in many patients, but PD issues with predominantly TNF-independent disease mechanisms may also play a role.

4. Immunogenicity of TNF antagonists may cause hypersensitivity reactions, both local and systemic. Immune-complex diseases with severe and even lethal vascular involvement have been reported.

5. Determining optimal therapy after drug failure is complicated. The current strategy of initial dose escalation followed by change to other TNF antagonists may lead to irreversible tissue damage while searching for an effective second drug, and it carries a high cost.

6. Theranostics, i.e., monitoring circulating levels of drug and ADA, appears to be essential for optimal and cost-effective interventions, and may prevent adverse reactions.

7. Assays should be designed to mimic the *in vivo* situation and report functionality of both drugs (TNF-α neutralization) and ADA (drug neutralization).

8. Solid phase binding assays,

e.g., ELISA, are considered inappropriate for safe therapeutic guidance as they do not reveal the functions of drugs and ADA, and their artificial setup has severe limitations in clinical use.

9. Screening for ADA is now required for marketing of all new biological drugs, including biosimilars (European Medicines Agency and U.S. FDA). Despite this, monitoring individual patients for ADA is not yet part of routine clinical practice.

Acknowledgments

I am indebted to the many colleagues who over the years have contributed to the basic and clinical research discussed in this article.

Disclosure

Within the last three years, Klaus Bendtzen has served as a speaker for Pfizer, Roche, Novo-Nordisk, Bristol-Meyers Squibb, and Biomonitor, and owns stocks in Novo-Nordisk and Biomonitor.

References

Aarden L, Ruuls SR, Wolbink G. Immunogenicity of anti-tumor necrosis factor antibodies - toward improved methods of anti-antibody measurement. *Curr Opin Immunol* 20(4):431-435, 2008.

Afif W, Loftus EVJ, Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 105(5):1133-1139, 2010.

Aikawa NE, JF dC, Silva CAA, Bonfa E. Immunogenicity of anti-TNF-alpha agents in autoimmune diseases. *Clin Rev Allergy Immunol* 38:82-89, 2010.

Ainsworth MA, Bendtzen K, Brynskov J. Tumor necrosis factor-alpha binding capacity and anti-infliximab antibodies measured by fluid-phase radioimmunoassays as predictors of clinical efficacy of infliximab in Crohn's disease. *Am J Gastroenterol* 103:944-948, 2008.

Allez M, Karmiris K, Louis E, Van Assche G, Ben-Horin S, Klein A, Van der Woude J, Baert F, Eliakim R, Katsanos K, Brynskov J, Steinwurz F, Danese S, Vermeire S, Teillaud J-L, Lémann M, Chowers Y. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. *J Crohn's Colitis* 4(4):355-366, 2010.

Assasi N, Blackhouse G, Xie F, Marshall JK, Irvine EJ, Gaebel K, Robertson D, Campbell K, Hopkins R, Goeree R. Patient outcomes after anti TNF-alpha drugs for Crohn's disease. *Expert Rev Pharmacoecon Outcomes Res* 10(2):163-175, 2010.

Atzeni F, Talotta R, Benucci M, Salaffi F, Cassinotti A, Varisco V, Battellino M, Ardizzone S, Pace F, Sarzi-Puttini P. Immunogenicity and autoimmunity during anti-TNF therapy. *Autoimmun Rev*, epub ahead of print, Nov. 30, 2012.

Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA,

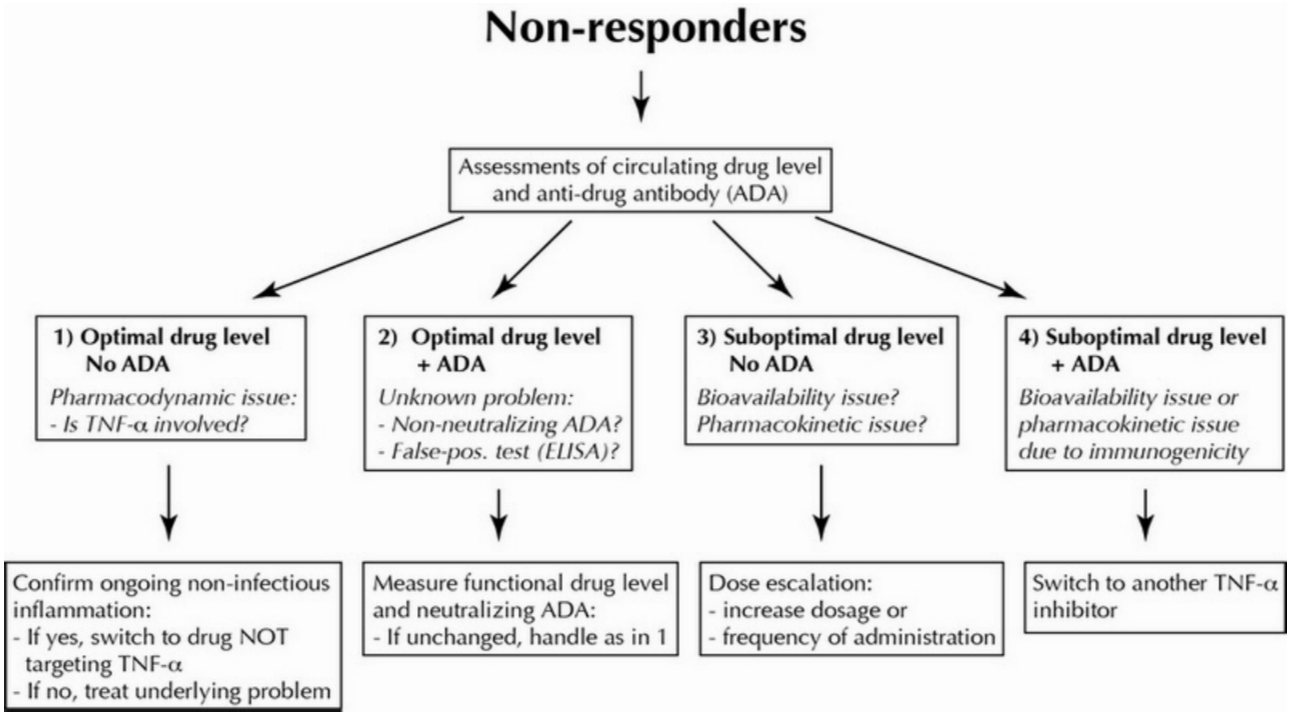

Figure 7. Decision algorithm for patients with primary and secondary response failure to TNF-α antagonists. Four scenarios encountered in patients with insufficient clinical response to TNF antagonists. Serum trough levels of functionally active drug and drug-neutralizing ADA are measured. Relevant cut-off levels for both drug and ADA depend on pre-determined clinical validation. Adapted with permission from *Scandinavian Journal of Gastroenterology* (Bendtzen *et al*., 2009).

Lems WF, Twisk JW, Dijkmans BA, Aarden L, Wolbink GJ. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* 305(14):1460-1468, 2011.

Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther* 2011.

Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor Infliximab. *Arthritis Rheum* 54:3782-3789, 2006.

Bendtzen K, Ainsworth M, Steenholdt C, Thomsen OO, Brynskov J. Individual medicine in inflammatory bowel disease: monitoring bioavailability, pharmacokinetics and immunogenicity of anti-tumour necrosis factor-alpha antibodies. *Scand J Gastroenterol* 44(7):774-781, 2009.

Bendtzen K. Editorial: Is there a need for immunopharmacological guidance of anti-TNF therapies? *Arthritis Rheum* 63(4):867-870, 2011.

Bendtzen K, Svenson M. Enzyme immunoassays and radioimmunoassays for quantification of anti-TNF biopharmaceuticals and anti-drug antibodies. In: *Detection and Quantification of Antibodies to Biopharmaceuticals. Practical and Applied Considerations.* Tovey MG, ed. pp 83-101. John Wiley & Sons, Inc., West Sussex, UK, 2011.

Bendtzen K. Anti-TNF-α biotherapies: perspectives for evidence-based personalized medicine. *Immunotherapy* 4(11):1167-1179, 2012.

Brunasso AM, Puntoni M, Massone C. Drug survival rates of biologic treatments in patients with psoriasis vulgaris. *Br J Dermatol* 166(2):447-449, 2012.

Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: A critical systematic review. *Inflamm Bowel Dis* 15:1264-1275, 2009.

Chaparro M, Guerra I, Munoz-Linares P, Gisbert JP. Systematic review: antibodies and anti-TNF-alpha levels in inflammatory bowel disease. *Aliment Pharmacol Ther*, epub ahead of print, Mar. 22, 2012.

Cheifetz A, Mayer L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. *Mt Sinai J Med* 72(4):250-256, 2005.

Colombel JF, Feagan BG, Sandborn WJ, Van Assche G, Robinson AM. Therapeutic drug monitoring of biologics for inflammatory bowel disease. *Inflamm Bowel Dis* 18(2):349-358, 2012.

Danese S, Colombel JF, Reinisch W, Rutgeerts PJ. Review article: infliximab for Crohn's disease treatment -- shifting therapeutic strategies after 10 years of clinical experience. *Aliment Pharmacol Ther* 33(8):857-869, 2011.

De Groot AS, Scott DW. Immunogenicity of protein therapeutics. *Trends Immunol* 28(11):482-490, 2007.

Desai D, Brightling C. TNF-alpha antagonism in severe asthma? *Recent Pat Inflamm Allergy Drug Discov* 4(3):193-200, 2010.

Descotes J, Gouraud A. Clinical immunotoxicity of therapeutic proteins. *Expert Opin Drug Metab Toxicol* 4(12):1537-1549, 2008.

Dubey S, Kerrigan N, Mills K, Scott DG. Bronchospasm associated with anti-TNF treatment. *Clin Rheumatol* 28(8):989-992, 2009.

Ebert EC, Das KM, Mehta V, Rezac C. Non-response to infliximab may be due to innate neutralizing anti-tumour necrosis factor-alpha antibodies. *Clin Exp Immunol* 154:325-331, 2008.

Emery P, Fleischmann RM, Moreland LW, Hsia EC, Strusberg I, Durez P, Nash P, Amante EJ, Churchill M, Park W, Pons-Estel BA, Doyle MK, Visvanathan S, Xu W, Rahman MU. Golimumab, a human anti-tumor necrosis factor alpha monoclonal antibody, injected subcutaneously every four weeks in methotrexate-naive patients with active rheumatoid arthritis: Twenty-four-week results of a phase III, multicenter, randomized, double-blind, placebo-controlled study of golimumab before methotrexate as first-line therapy for early-onset rheumatoid arthritis. *Arthritis Rheum* 60(8):2272-2283, 2009.

Emery P. Optimizing outcomes in patients with rheumatoid arthritis and an inadequate response to anti-TNF treatment. *Rheumatology (Oxford)* 51(Suppl 5):v22-v30, 2012.

European Medicines Agency (EMA). Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003946.pdf). pp1-18, 2007.

Fefferman DS, Farrell RJ. Immunogenicity of biological agents in inflammatory bowel disease. *Inflamm Bowel Dis* 11(5):497-503, 2005.

Furst DE, Keystone EC, Braun J, Breedveld FC, Burmester GR, De Benedetti F, Dorner T, Emery P, Fleischmann R, Gibofsky A, Kalden JR, Kavanaugh A, Kirkham B, Mease P, Sieper J, Singer NG, Smolen JS, Van Riel PL, Weisman MH, Winthrop K. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2010. *Ann Rheum Dis* 70(Suppl 1):i2-i36, 2011.

Garces S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. *Ann Rheum Dis*, epub ahead of print, Dec. 6, 2012.

Hart MH, de Vrieze H, Wouters D, Wolbink GJ, Killestein J, de Groot ER, Aarden LA, Rispens T. Differential effect of drug interference in immunogenicity assays. *J Immunol Methods* 372:196-203, 2011.

Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods* 36:3-10, 2005.

Jamnitski A, Bartelds GM, Nurmohamed MT, van Schouwenburg PA, van Schaardenburg D, Stapel SO, Dijkmans BA, Aarden L, Wolbink GJ. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. *Ann Rheum Dis* 70:284-288, 2010.

Jamnitski A, Krieckaert CL, Nurmohamed MT, Hart MH, Dijkmans BA, Aarden L, Voskuyl AE, Wolbink GJ. Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. *Ann Rheum Dis* 64(12):3850-3855, 2012.

Jemec GB. Predicting response to anti-TNF-alpha treatment in Hidradenitis suppurativa. *Br J Dermatol* 168(2):233, 2013.

Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, Claes K, Coopman T, Van Schuerbeek N, Van Assche G, Vermeire S, Rutgeerts P. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 137(5):1628-1640, 2009.

Korswagen LA, Bartelds GM, Krieckaert CL, Turkstra F, Nurmohamed MT, van Schaardenburg D, Wijbrandts CA, Tak PP, Lems WF, Dijkmans BA, van Vugt RM, Wolbink GJ. Venous and arterial thromboembolic events in adalimumab-treated patients with antiadalimumab antibodies: a case series and cohort study. *Arthritis Rheum* 63(4):877-883, 2011.

Krieckaert C, Rispens T, Wolbink G. Immunogenicity of biological therapeutics: from assay to patient. *Curr Opin Rheumatol* 24(3):306-311, 2012.

Krieckaert CL, Bartelds GM, Lems WF, Wolbink GJ. The effect of immunomodulators on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies: a review. *Arthritis Res Ther* 12(5):217, 2010.

Kromminga A, Schellekens H. Antibodies against erythropoietin and other protein-based therapeutics: An overview. *Ann N Y Acad Sci* 1050:257-265, 2005.

Lallemand C, Kavrochorianou N, Steenholdt C, Bendtzen K, Ainsworth MA, Meritet JF, Blanchard B, Lebon P, Taylor P, Charles P, Alzabin S, Tovey MG. Reporter gene assay for the quantification of the activity and neutralizing antibody response to TNFalpha antagonists. *J Immunol Methods* 373(1-2):229-239, 2011.

Langford CA. Drug insight: anti-tumor necrosis factor therapies for the vasculitic diseases. *Nat Clin Pract Rheumatol* 4(7):364-370, 2008.

Lecluse LL, Driessen RJ, Spuls PI, de Jong EM, Stapel SO, van Doorn MB, Bos JD, Wolbink GJ. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. *Arch Dermatol* 146(2):127-132, 2010.

Lin J, Ziring D, Desai S, Kim S, Wong M, Korin Y, Braun J, Reed E, Gjertson D, Singh RR. TNFalpha blockade in human diseases: an overview of efficacy and safety. *Clin Immunol* 126(1):13-30, 2008.

Makol A, Grover M, Guggenheim C, Hassouna H. Etanercept and venous thromboembolism: a case series. *J Med Case Reports* 4:12, 2010.

Mulleman D, Meric JC, Paintaud G, Ducourau E, Magdelaine-Beuzelin C, Valat JP, Goupille P. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. *Arthritis Res Ther* 11(6):R178, 2009.

Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther* 91(4):635-646, 2012.

Palekar-Shanbhag PS, Jog SV, Gaikwad SS, Chogale MM. Theranostics for cancer therapy. *Curr Drug Deliv*, epub ahead of print, Dec. 31, 2012.

Petitpain N, Gambier N, Wahl D, Chary-Valckenaere I, Loeuille D, Gillet P. Arterial and venous thromboembolic events during anti-TNF therapy: a study of 85 spontaneous reports in the period 2000-2006. *Biomed Mater Eng* 19(4-5):355-364, 2009.

Radstake TR, Svenson M, Eijsbouts AM, van den Hoogen FH, Enevold C, van Riel PL, Bendtzen K. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 68(11):1739-1745, 2009.

Shankar G, Shores E, Wagner C, Mire-Sluis A. Scientific and regulatory considerations on the immunogenicity of biologics. *Trends Biotechnol* 24(6):274-280, 2006.

St. Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, Keystone EC. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis. Results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 46:1451-1459, 2002.

Steenholdt C, Svenson M, Bendtzen K, Thomsen O, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 34(1):51-58, 2011a.

Steenholdt C, Bendtzen K, Brynskov J, Thomsen OO, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 46(3):310-318, 2011b.

Steenholdt C, Al-khalaf M, Brynskov J, Bendtzen K, Thomsen O, Ainsworth MA. Clinical implications of variations in anti-infliximab antibody levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 18(12):2209-2217, 2012.

Steenholdt C, Brynskov J, Thomsen O, Munck LK, Fallingborg J, Christensen LA, Pedersen G, Kjeldsen J, Jacobsen BA, Oxholm AS, Kjellberg J, Bendtzen K, Ainsworth MA. Treatment of secondary infliximab failure in Crohn's disease based on serum levels of infliximab and antibodies against infliximab: the Danish study of Optimizing Infliximab Therapy in Crohn's Disease (DO IT CROHN) randomized clinical trial. *Digestive Disease Week*, Orlando, Florida, USA, 2013.

Strand V, Kimberly R, Isaacs JD. Biologic therapies in rheumatology: lessons learned, future directions. *Nat Rev Drug Discov* 6(1):75-92, 2007.

Svenson M, Geborek P, Saxne T, Bendtzen K. Monitoring patients treated with anti-TNF-alpha biopharmaceuticals - assessing serum infliximab and anti-infliximab antibodies. *Rheumatology* 46:1828-1834, 2007.

Tanaka Y. Next stage of RA treatment: is TNF inhibitor-free remission a possible treatment goal? *Ann Rheum Dis* 72(Suppl 2):ii124-ii127, 2013.

Tangri S, Mothe BR, Eisenbraun J, Sidney J, Southwood S, Briggs K, Zinckgraf J, Bilsel P, Newman M, Chesnut R, Licalsi C, Sette A. Rationally engineered therapeutic proteins with reduced immunogenicity. *J Immunol* 174(6):3187-3196, 2005.

Ternant D, Aubourg A, Magdelaine-Beuzelin C, Degenne D, Watier H, Picon L, Paintaud G. Infliximab pharmacokinetics in inflammatory bowel disease patients. *Ther Drug Monit* 30(4):523-529, 2008.

van den Bemt BJ, den Broeder AA, Wolbink GJ, Hekster YA, van Riel PL, Benraad B, van den Hoogen FH. Anti-infliximab antibodies are already detectable in most patients with rheumatoid arthritis halfway through an infusioncycle: an open-label pharmacokinetic cohort study. *BMC Musculoskelet Disord* 12:12, 2011.

van der Maas A, van den Bemt BJ, Wolbink GJ, van den Hoogen FH, van Riel PL, den Broeder AA. Low infliximab serum trough levels and anti-infliximab antibodies are prevalent in rheumatoid arthritis patients treated with infliximab in daily clinical practice: results of an observational cohort study. *BMC Musculoskelet Disord* 13(1):184, 2012.

van Schouwenburg PA, Krieckaert CL, Nurmohamed M, Hart M, Rispens T, Aarden L, Wouters D, Wolbink GJ. IgG4 production against adalimumab during long term treatment of RA patients. *J Clin Immunol* 32(5):1000-1006, 2012.

Wang SL, Ohrmund L, Hauenstein S, Salbato J, Reddy R, Monk P, Lockton S, Ling N, Singh S. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods* 382(1-2):177-88, 2012.

West RL, Zelinkova Z, Wolbink GJ, Kuipers EJ, Stokkers PC, Van der Woude CJ. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. *Aliment Pharmacol Ther* 28(9):1122-1126, 2008.

Wijbrandts CA, Dijkgraaf MG, Kraan MC, Vinkenoog M, Smeets TJ, Dinant H, Vos K, Lems WF, Wolbink GJ, Sijpkens D, Dijkmans BA, Tak PP. The clinical response to infliximab in rheumatoid arthritis is in part dependent on pretreatment tumour necrosis factor alpha expression in the synovium. *Ann Rheum Dis* 67(8):1139-1144, 2008.

Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BA, Aarden L. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 64(5):704-707, 2005.

Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 54(3):711-715, 2006.

Wolbink GJ, Aarden LA, Dijkmans BA. Dealing with immunogenicity of biologicals: assessment and clinical relevance. *Curr Opin Rheumatol* 21(3):211-215, 2009.

Yanai H, Hanauer SB. Assessing response and loss of response to biological therapies in IBD. *Am J Gastroenterol* 106:685-698, 2011.