Review

Immunogenicity of anti-tumour necrosis factor drugs in rheumatic diseases

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ABSTRACT

Despite the significant advantages in clinical practice associated with TNF-inhibitors, a loss of response over time is sometimes observed, in some cases possibly due to immunogenicity, i.e. the development of antibodies direct against the drug. This review evaluates the immunogenicity of different anti-TNF agents, and discusses its effects on efficacy and safety. Available evidence indicates that all anti-TNF drugs may induce an immune response. However, the variation in the occurrence of anti-drug antibodies, as well as the variation in the impact of antibodies on the efficacy and safety, can be explained by drug conformation itself, use of concomitant immunosuppressants and differences in dosing regimen and route of administration. The association between the development of anti-drug antibodies and low drug serum concentrations is clinically relevant since it is likely related to low response. Strict monitoring of neutralising antibodies might be useful for tailoring therapeutic strategy. There is no evidence of cross-reactivity among different drugs: immunogenicity (the development of specific anti-drug antibodies to one TNF inhibitor) does not seem to affect the effectiveness of another anti-TNF agents; therefore, switching to another drug of the same class might be effective in patients who have developed anti-drug antibodies to a TNF inhibitor.

Introduction

Cytokines are important molecular targets for therapy in rheumatic diseases. Among these inflammatory mediators, TNF has emerged as a key target in inflammatory arthritides. The available drugs that target TNF are monoclonal antibodies (MAb) or derivatives thereof; they include chimeric, humanised or fully human MAb and antibody fragments (Fc or Fab) (1). Anti-TNF agents currently approved for treatment of rheumatic diseases include the MAbs infliximab (IFX), adalimumab (ADA) and golimumab (GLM), the fusion protein etanercept (ETN) and the PEGylated Fab fragment certolizumab pegol (CZP) (2). Despite the significant advantages in clinical practice associated with these drugs, a loss of response over time is sometimes observed; in some cases immunogenicity, *i.e.* the development of antibodies against the drug, can be implicated in the reduced drug efficacy (3). IFX is a chimeric monoclonal anti-TNF comprising the constant region of human immunoglobulin G1 kappa (IgG_{1K}) with mouse variable heavy and light chain domains, conferring high affinity and neutralising capacity. The murine sequences account for 25% of the whole structure. Because the murine amino acid sequences differ from their human counterpart, IFX may induce human anti-mouse antibodies directed to the murine variable binding region (complementarity-determining regions and framework regions) of the antibody.

Attempts to reduce immune response to the chimeric antibody include removal of murine components and development of fully human antibodies: ADA and GLM are fully human IgG_{1K} antibodies with anti-TNF activity. However, fully human MAbs still have some immunogenicity and human anti-human antibodies have been detected in patients treated with these drugs. ETA is an engineered dimeric p75 TNF-receptor fusion protein linked to the Fc portion of human IgG1; it only includes human amino acid sequences. The newest anti-TNF agent is CZP, a PEGylated Fab fragment of humanised anti-TNF. Fab fragments are generally less immunogenic than whole antibodies and the addition of polyethylene glycol (PEG) could further

Table I. Incidence of anti-infliximab antibodies in rheumatic diseases.

Disease	n.	IFX dosage	Follow-up	Method	% Ab	Neutralising (y/n)	Effect on clinical response	Reference
RA	87	1, 3, 10 mg/kg 0, 2, 6, q8w	26 week	ELISA	17.4%	n.r.	NR	Maini *et al*. (5)
RA	51	3 mg/kg 0, 2, 6, q8w	n.r.	ELISA	39%	↓ IFX levels	NR	Haraoui *et al*. (6)
RA	51	3 mg/kg 0, 2, 6, q8w	1 year	RIA	43%	↓ IFX levels	Yes	Wolbink *et al*. (7)
RA	106	3 mg/kg 0, 2, 6, q8w	6 months	RIA	44%	Yes	Yes	Bendzen *et al*. (8)
RA	35	3 mg/kg 0, 2, 6, q8w	6 months	RIA	51.4%	↓ IFX levels	Yes	Radstake *et al*. (9)
RA	64	3 mg/kg 0, 2, 6, q8w	n.r.	ELISA	12.5%	↓ IFX levels	association with acquired drug resistance	Finckh *et al*. (10)
RA	85	3 mg/kg 0, 2, 6, q8w	5.9 years*	ELISA	32.9%	↓ IFX levels	Yes	Pascual-Salcedo *et al*. (11)
RA	40	3 mg/kg 0, 2, 6, q8w	54 weeks	ELISA	35%	↓ IFX levels	Yes	Hoshino *et al*. (13)
SpA, RA	108 (91, 17)	3 mg–5 mg/kg 0, 2, 6, q8w	n.r.	ELISA	19% (15%, 41%)	↓ IFX levels	NR	Ducourau *et al*. (12)
SpA	56	5 mg/kg 0, 2, 6, q8w	4 years	ELISA	25.5%	↓ IFX levels	Yes	Plasencia *et al*. (15)
PsA	100	5 mg/kg 0, 2, 6, q8w	66 weeks	n.r.	15.4%	n.r.	NR	Kavanaugh *et al*. (16)
AS	38	5 mg/kg 0, 2, 6, q8w	54 weeks	RIA	29%	↓ IFX levels	Yes	de Vries *et al*. (17)
JIA	21	3–5 mg/kg 0, 2, 6, q8w	n.r.	ELISA	43%	↓ IFX levels	NR	Kosmač *et al*. (18)

AS: ankylosing spondylitis; ELISA: enzyme-linked immunosorbent assay; IFX: infliximab; JIA: juvenile idiopathic arthritis; NR: not reported; PsA: psoriatic arthritis; RA: rheumatoid arthritis; RIA: radioimmunoassay. *: mean follow-up.

Table II. Incidence of anti-adalimumab antibodies in rheumatic diseases.

Disease	n.	ADA dosage	Follow-up	Method	% Ab	Neutralising (y/n)	Effect on clinical response	Reference
RA	217	20, 40, 80 mg eow	24 weeks	ELISA	1%	n.r.	NR	Weinblatt *et al*. (19)
RA	419	20 mg weekly or 40 mg eow	52 weeks	ELISA	0.72%	n.r.	NR	Keystone *et al*. (20)
RA	434	20 or 40 mg weekly or eow	26 weeks	ELISA	12%	n.r.	Yes	van de Putte *et al*. (21)
RA	121	40 mg sc eow	28 weeks	RIA	17%	↓ ADA levels	Yes	Bartelds *et al*. (22)
RA	15	40 mg sc eow	18 months	ELISA	87%	n.r.	Yes	Bender *et al*. (23)
RA	34	40 mg sc eow	6 months	RIA	29%	↓ ADA levels	Yes	Radstake *et al*. (9)
RA	30	40 mg sc eow	28 weeks	ABT/PIA	17/70%	n.r.	NR	van Schowenburg *et al*. (24)
RA	235	40 mg sc eow	28 weeks	RIA	20%	↓ ADA levels	Yes	Bartelds *et al*. (25)
RA	272	40 mg sc eow	3 years	RIA	28%	↓ ADA levels	Yes	Bartelds *et al*. (26)
RA	30	40 mg sc eow	56 weeks	ELISA	4.9%	n.r.	No	van der Bijl (27)
PsA	22	40 mg sc eow	12 months	RIA	18%	↓ ADA levels	Yes	van Kujik *et al*. (29)
AS	35	40 mg sc eow	6 months	RIA	31%	↓ ADA levels	Yes	De Vries *et al*. (28)
JIA	20	20 or 40 mg eow	24 weeks	ELISA	16%	↓ ADA levels	NR	Imagawa *et al*. (30)

ABT: antigen binding test; ADA: adalimumab; AS: ankylosing spondylitis; ELISA: enzyme-linked immunosorbent assay; eow: every other week; PIA: pH-shift-anti-idiotype ABT; NR: not reported; PsA: psoriatic arthritis; RA: rheumatoid arthritis; RIA: radioimmunoassay.

reduce the immunogenicity (4). Given that they are exogenous proteins, all the above-mentioned drugs may be immunogenic. However, the development of anti-drug antibodies and their impact on efficacy and safety depend on several factors, which, in addition to extent of humanisation, include dosing regimen and route of administration (3).

This narrative review evaluates the differential immunogenicity of currently approved anti-TNF agents, and discusses the relevance of immunogenicity on efficacy and safety.

Search criteria

Key studies for inclusion were identified by a MEDLINE search, based on several interrelated queries (*e.g.* "immunogenicity" OR "anti-drug" AND "anti TNF" AND "rheumatoid arthritis" OR "psoriatic arthritis" OR "ankylosing spondylitis" OR "juvenile idiopathic arthritis"). Restrictions in terms of time were not applied. Only studies in English were considered. The articles retrieved were chosen according to their relevance, as judged by the authors. The search results were then supplemented by browsing the reference lists of identified articles, and by including other documents suggested by authors' experience.

Incidence of anti-drug antibodies

The incidence of anti-drug antibodies with anti-TNF agents are summarised in Tables I–V.

Infliximab

Occurrence of anti-IFX antibodies has been observed in 12–60% of patients receiving treatment for rheumatoid arthritis (RA) and anti-drug antibodies

Table III. Incidence of anti-etanercept antibodies in rheumatic diseases.

Disease	n.	ETA dosage	Follow-up	Method	% Ab	Neutralising (y/n)	Effect on clinical response	Reference
RA	367	25x2 or 50/week	16 week	n.r.	3%	no	No	Keystone *et al*. (32)
RA	222	50 mg/week	24 weeks	ELISA	5.6%	no*	No	Dore *et al*. (31)
RA	40	25 mg x2/week	32 weeks	RIA	0%	NA	NA	Hoshino *et al*. (13)
RA	549	25 mg x2/week	193 weeks	ELISA	5%	n.r.	n.r.	Klareskog *et al*. (33)
RA	292	50 mg/week	6 months	RIA/ELISA/ IgG4 ABT	0%	NA	NA	Jamintski *et al*. (34)
PsA	104	25 mg x2/week	24 weeks	n.r.	0%	NA	NA	Mease *et al*. (36)
AS	53	25 mg x2/week	24 weeks	RIA	0%	NA	NA	deVries *et al*. (35)

ABT: antigen binding test; AS: ankylosing spondylitis; ELISA: enzyme-linked immunosorbent assay; ETA: etanercept; NA: not applicable; NR: not reported; PsA: psoriatic arthritis; RA: rheumatoid arthritis; RIA: radioimmunoassay.
*neutralising assay: sera are defined as neutralising if able to reduce drug concentration by at least 50%.

appear mostly between the third and sixth month of therapy (5-13) (Table I). A recent publication has investigated the kinetics of anti-drug emergence in serum of 27 RA patients collected at 5 time-points during a single IFX cycle (one hour before the first infusion, one hour after, at 50% and 75% of the cycle and at the time of the subsequent infusion) (14). The mean interval between the two IFX infusions was 6.8±2 weeks: about 70% of anti-IFX antibodies was already detectable halfway through an infusion cycle, and correlated with IFX serum levels (14). Data on IFX immunogenicity in ankylosing spondylitis (AS) and spondyloarthritis (SpA) are scant (12, 15-17): anti-IFX antibodies have been detected in 29% of 38 patients with AS treated with 5 mg/kg IFX on weeks 0, 2, 6 and every 6 weeks thereafter (17). Interestingly, the presence of anti-IFX antibodies was associated with the absence of HLA-B27, a marker of AS susceptibility (17). A recent study in 94 patients with SpA observed for over more than 4 years has confirmed previous findings, with 25.5% of patients developing antibodies during a mean time of IFX exposure of 6.99 years (15). Anti-IFX antibodies have also been detected in 43% of paediatric patients with juvenile idiopathic arthritis (JIA) (18). In most of the studies, the emergence of anti-IFX antibodies was associated to a decrease in serum trough level of the drug (Table I).

Adalimumab

The emergence of anti-ADA antibodies in RA patients has been investigated in short-term and long-term studies (28 weeks to 3 years) and ranges from less than 1% to 87% (Table II) (9, 19-27); anti-drug antibodies have been identified as early as 4 weeks after treatment initiation in up to 10% of patients (22, 26). The rate of anti-ADA antibodies in psoriatic arthritis (PsA) and AS patients was estimated to be 18% over a 12-month follow-up and 31% over 6 months, respectively (28, 29). Anti-ADA antibodies have also been detected in 16% of JIA patients (30). The emergence of anti-ADA antibodies has been associated to a decrease in drug serum levels (9, 22, 25, 26, 28-30).

Etanercept

Anti-ETA antibodies have been described in 3–5.6% of RA patients treated with ETA 25 mg twice weekly or 50 mg once weekly, with a maximum follow-up of 5 years; antibodies were transient and non-neutralising (Table III) (13, 31-34). No anti-drug antibodies have been observed in patients treated with ETA for PsA and AS (35, 36). No significant effects in terms of clinical response, side effects, injection site reactions and allergic reactions have been associated with anti-ETA antibodies (25-27, 29).

Certolizumab and golimumab

Data on the immunogenicity of CZP and GLM derive only from clinical trials, and are still scant. Anti-CZP antibodies have been detected in 5–6.4% of RA treated patients taking CZP as monotherapy or with concomitant methotrexate (MTX); among anti-CZP positive patients, 2–8.1% developed neutralising antibodies (Table IV) (37-40).

The reported emergence of anti-GLM in RA patients ranges from 2 to 6.5% (Table V) (41-48). A higher proportion of patients receiving GLM as monotherapy developed anti-drug antibodies (9–13.5%) (41, 42, 45); GLM concentration was lower in anti-GLM positive patients (41). Anti-GLM have been detected in 4.6–4.9% patients with PsA and 1.4–4.1% patients with AS (49-52). Anti-GLM positive patients did not receive concomitant MTX and generally had low drug concentration (51).

Factor influencing drug immunogenicity

Several different factors belonging both to the drug administered (*i.e*. degree of humanisation, dosing regimen, route of administration) or the patient (*i.e*. concomitant use of immunosuppressant, disease activity) may influence the development of anti-drug antibodies.

Concomitant therapies

Immunosuppressant therapy is a key factor affecting development of anti-drug antibodies; however, it is still unclear whether immunosuppressant co-administration reduces immunogenicity (3). Most of the studies investigating the emergence of anti-IFX antibodies in patients with rheumatic disease have not demonstrated any effect of concomitant MTX on anti-IFX antibody development (6-8, 10, 11, 53). In a 26-week clinical trial of IFX in 101 RA patients, co-administration of

Table IV. Incidence of anti-certolizumab antibodies in rheumatoid arthritis.

n.*	CZP dosage	Follow-up	Method	% Ab	Neutralising (y/n)	Effect on clinical response	Reference
783	400 w 0, 2, 4 than 200 or 400 q2w	52 weeks	ELISA	6.4	n.r.	n.r.	Keystone *et al*. (37)
492	400 w 0, 2, 4 than 200 or 400 q2w	24 weeks	n.r.	5.1%	yes (36%)	n.r.	Smolen *et al*. (39)
111	400 mg q4w	24 weeks	ELISA	8.1%	yes	Yes	Fleischmann *et al*. (38)
126	400 mg q4w	24 weeks	n.r.	5%	n.r.	n.r.	Choy *et al*. (40)

CZP: certolizumab; ELISA: enzyme-linked immunosorbent assay; q2w: every 2 weeks; q4w: every 4 weeks.
*number of patients exposed to CZP during clinical trials.

low-dose MTX lowered the incidence of antibodies from 53%, 21% and 7% to 15%, 7% and 0% in RA patients receiving 1, 3 and 10 mg/kg IFX, respectively (5). Similarly, two studies in patients with SpA patients have demonstrated a lower incidence of anti-IFX antibodies in patients receiving MTX (12, 15). Moreover, Pascual-Salcedo *et al*. demonstrated that the combined therapy was associated to lower levels of anti-drug antibodies (11). More consistent data on immunosuppressant effects in preventing anti-IFX antibodies come from patients with Crohn's disease; however, this information is out of the scope of this review and will not be discussed.

Concomitant MTX has been associated with a lower production of anti-ADA antibodies in a few studies (22, 23, 26). In 272 patients with RA who were followed in a long-term study, those who later developed anti-ADA antibodies received concomitant MTX less often (52% *vs*. 82%, $p<0.0001$), and more often required no concomitant disease-modifying anti-rheumatic drug (DMARD) (26). In the same population, the dose-effect of MTX administration on reduction of immunogenicity has been recently demonstrated (54).

The role of MTX on reducing anti-drug antibody emergence has been related to its immunosuppressive properties. Drug-induced tolerance has been studied in mice and could be responsible for the effect in humans (55, 56). However, MTX also exerts an anti-inflammatory effect, decreasing TNF produced by inflamed tissue; thus, reduced circulating TNF levels might consume fewer anti-TNF antibodies and the resulting higher circulating drug levels may induce human anti-drug antibody formation (57). However, other immunosuppressive drugs have not shown a clear association with lower levels of anti-drug antibodies in rheumatic disease (8, 10, 22). In a small study on 20 RA patients, 100% of patients with anti-ADA antibody received ADA as monotherapy, while anti-ADA antibodies were detected in 72.7% of those treated with combination therapy (ADA + DMARDs) (23).The effect of corticosteroid therapy on anti-drug development seems less evident (6, 8). A study designed to address the effect of anti-IFX antibodies on the need for dose escalation in RA patients demonstrated that a lower corticosteroid dose correlated with increased antibody production (6). In another study, concomitant corticosteroids did not affect antibody emergence (8).

Therapeutic regimen

Dose and therapeutic regimen may play a central role in the anti-IFX antibody development. Interestingly, a lower IFX dose was associated with a higher incidence of anti-IFX antibodies with 57%, 25% and 10% of patients who received 1, 3 and 10 mg/kg, testing positive; this is likely due to the direct immunosuppressive effect of anti-TNF treatment (5). While the "episodic" regimen employed for Crohn's disease seems to be more immunogenic (58), in rheumatic diseases a continuous maintenance schedule is utilised. Data on effect of therapeutic regimen and dosage can be gathered from the observation of patients needing a dose escalation for inadequate clinical response. In a study conducted by de Vries *et al*., 11 out of 38 AS patients tested positive for anti-IFX after a follow-up of 54 weeks. Among these patients, 9 (75%) needed dose escalation for insufficient response; however, the increased IFX dose was not associated with an increase in IFX trough levels, nor a significant decrease in anti-IFX antibodies levels (17). The emergence of anti-IFX in a high percentage of AS patients treated with IFX suggests that dose augmentation might induce more frequent antibody development. On the contrary, two different studies in RA patients treated with a TNF inhibitor demonstrated a decrease of anti-drug antibodies after dose escalation or an increase in dosing frequency (3, 22). Wolbink *et al*. studied the rate of anti-IFX antibodies at 1 year in 51 patients with RA and observed that 3 out of 22 anti-drug positive patients needed an increase of IFX dose (3). Increasing the IFX dose led to reduction of anti-IFX below the detection level (3). In another study, anti-ADA antibodies were no longer detectable after increasing dosing frequency in 10 out of 121 RA patients who were followed-up for 28 weeks (22). Finally, in a recently published prospective, observational, longitudinal study in 147 RA patients, a 5.2 relative risk (RR) of anti-IFX emergence after drug administration interval increase and a 4.9 RR of disappearance after interval decrease were demonstrated (53).

The disappearance of anti-drug antibodies after dose escalation could be explained by the induction of immunotolerance (3). High-dose tolerance is an immunologic phenomenon that can explain the inverse dose-response effect in immunogenicity of TNF antagonists. Interestingly, in some anti-IFX positive patients with inadequate response, higher doses of drug resulted in decreased levels of anti-IFX antibodies

Table V. Incidence of anti-GLM antibodies in rheumatic diseases.

Disease	n.*	GLM dosage	Follow-up	Method	% Ab	Neutralising (y/n)	Effect on clinical response	Reference
RA	107	50 or 100 mg sc q2 or q4w	48 weeks	ELISA	6.5%	↓ GLM levels	NR	Kay *et al*. (41)
RA	315	50 or 100 mg sc q4w	24 weeks	ELISA	6.3%	NR	NR	Emery *et al*. (42)
RA	236	50 or 100 mg sc q4w	24 weeks	ELISA	2.1%	NR	NR	Keystone *et al*. (43)
RA	264	50 or 100 mg sc q4w	24 weeks	ELISA	3%	NR	NR	Smolen *et al*. (44)
RA	529	2 or 4 mg/kg iv q12w	24 weeks	ELISA	5%	NR	Yes	Kremer *et al*. (45)
RA	173	50 or 100 mg sc q4w	24 weeks	ELISA	0%	NA	NA	Tanaka *et al*. (46)
RA	440	2 mg/kg iv w 0, 4, then q8w	24 weeks	ELISA	3%	↓ GLM levels	NR	Weinblatt *et al*. (47)
RA	33+16	100 mg sv *vs*. 2 mg/kg iv 0–12 w	30 weeks	ABT	6.25% *vs*. 0%	NR	NR	Zhuang *et al*. (48)
PsA	292	50 or 100 mg sc q4w (until w 20)	24 weeks	ELISA	4.6%	NR	NR	Kavanaugh *et al*. (49)
PsA	292	50 or 100 mg sc q4w (w 24–52)	52 weeks	NR	4.9%	Yes	NR	Kavanaugh *et al*. (50)
AS	278	50 or 100 mg sc q4w	24 weeks	ELISA	4.1%	↓ GLM levels	NR	Inman *et al*. (51)
AS	356**	50 or 100 mg sc q4w	104 weeks	ELISA	1.4%	NR	NR	Braun *et al*. (52)

ABT: antigen binding test; AS: ankylosing spondylitis; ELISA: enzyme-linked immunosorbent assay, i.v.: intravenous; NA: not applicable; NR: not reported; PsA: psoriatic arthritis; q2w: every 2 weeks; q4w: every 4 weeks; RA: rheumatoid arthritis; s.c.: subcutaneous. *number of patients exposed to GLM during clinical trials; **whole population.

(3). Alternatively, the increased drug availability might overload the capacity of the immune system to produce anti-drug antibodies (22). To discriminate between these two hypotheses, an assay capable of detecting anti-drug in the presence of drug is needed (24). However, in most patients, antibody titres increase during treatment and are only temporarily modulated by an increase in IFX dose (11).

Disease activity

Disease activity might influence the bioavailability and immunogenicity of monoclonal anti-TNF antibodies (3). This hypothesis is supported by the finding that patients with higher disease activity at baseline show lower bioactive drug levels and higher incidence of anti-drug antibodies (8, 26, 59). Among patients who developed anti-IFX and anti-ADA antibodies, higher baseline disease activity and C-reactive protein levels, longer disease duration and more frequent erosive disease have been reported (8, 26, 59).

How to measure anti-drug antibodies

To date, different methodological approaches have been utilised to investigate the emergence of anti-drug antibodies during short-term and long-term treatment in systemic inflammatory diseases; however a standardisation of these different assays is still lacking.

The assessment of the immunogenicity profile of biotherapeutics comprises the detection of antibodies in the serum by immunochemical assays (60). These include different types of enzyme-linked immunosorbent assay (ELISA) in which an IgG construct, bound to an immobilised TNF-α, is detected with rabbit or goat anti-human IgG-Fc antibody (60, 61). However, a lack of secondary reagents able to discriminate between "normal" Ig and "induced" anti-drug antibodies is an issue (62, 63). Human anti-human antibodies, such as the IgG rheumatoid factor (RF), could interfere in the quantification of anti-drug antibodies in solid phase ELISA (63). Attempts to minimise the effect of circulating antibodies on anti-drug testing have utilised different approaches. The use of $F(ab)'_2$ fragments of ADA or IFX for anti-drug antibody binding could give a highly specific response because binding of unspecific antibodies to the Fc portion of ADA or IFX is prevented (64). Moreover, when trying to detect anti-drug antibodies, a significant problem arises when the drug itself is an antibody. Excess serum MAb may also interfere with antibody binding to the immobilised biotherapeutic agent.

A two-site, bridging ELISA for the detection of anti-drug antibodies takes advantage of the monovalency of the two arms of IgG subclasses 1, 2 and 3, which allows crosslinking of the biotherapeutic agent coated onto ELISA plates to biotinylated circulating drug (11). However, bridging ELISA may underestimate the immunogenicity of a biotherapeutic because it does not recognise IgG4 class antibodies which are generated during long-term exposure to the drug. This is because of the functional monovalency of the IgG4 that renders the molecule unable to cross-link the same antigen (64). Using an antigen binging test (ABT) carried out with both protein A sepharose and IgG4-coupled sepharose capturing anti-ADA ^{125}I-labelled $F(ab)_2$, van Schowenburg *et al*. have recently confirmed an IgG4 response to ADA (65).

The radioimmunoassay (RIA) for detecting serum anti-IFX antibody levels exploits the fact that IFX comprises only kappa light chains and any radioactive complex extracted by anti-human lambda light chain antibodies would be IFX bound to antibody (8). The antigen-binding assay is based on capturing serum antibodies by a sepharose-bound reagent and subsequently measuring the antibody reactivity by incubation with ^{125}I-labelled Fab fragments of the biotherapeutic antibody (7). The RIA method has some advantages: it is a fluid-phase assay resembling the *in vivo* situation and the biotherapeutic agent used is not denatured by coating. Moreover, RIA is a functional assay proving the capacity of the drug to bind TNF, rather than simply revealing a protein, which may or may

not be functional. As the available monoclonal anti-TNF antibodies are human IgG_{1K} constructs, which do not react with anti-human lambda light-chain antibodies, RIA can be used to monitor antibodies to these compounds by binding radiolabelled drug to an anti-lambda light-chain affinity matrix, revealing the drug-lambda light-chain antibody complexes. As ETA does not contain light chains, both anti-lambda and -kappa light-chain antibodies may be used (66).

The immune response against a drug includes antibodies directed against epitopes localised within the hypervariable regions of heavy and light chains (*i.e* anti-idiotype, neutralising antibodies) and antibodies directed against allotypes, epitopes not localised in the antigen-binding region (*i.e*. anti-allotype). To be considered neutralising, anti-drug antibodies should be able to suppress binding of biotherapuetic to its target. Svenson *et al*. tested the neutralising effect of anti-IFX antibodies in fluid phase RIA by demonstrating the competitive effect of the addition of IFX to anti-IFX positive sera on ^{125}I-labelled-TNF binding (66). To confirm the neutralising nature of anti-IFX a Fab neutralisation assay has been performed by adding IFX Fab fragment previously incubated with patients' serum to immobilised recombinant human TNF in a competition ELISA (18).

Anti-drug antibody detection is particularly problematic if sera are taken shortly after drug administration; therapeutic MAbs have long circulation half-lives, often resulting in the presence of residual therapeutic agent in serum drawn for analysis. For example, IFX can affect antibody detection at concentrations as little as 2 mg/mL (8).When IFX is added to anti-IFX antibody positive sera, some patients become negative, suggesting a competitive effect; hence, anti-drug antibodies can be detected reliably only in patients who have no detectable serum IFX and accurate detection of anti-IFX may require a prolonged wash-out period (10).

A reporter-gene assay which can detect the neturalising activity of anti-drug antibodies has recently been developed (62). This method does not appear to be influenced by the presence of drug nor TNF (or other cytokines) in the tested samples. Briefly, a human eritroleukaemic cell line was transfected with Nuclear Factor κB (NFκB) regulated reported-gene (firefly luciferase – FL); the cells were then transfected with Renilla luciferase (RL) gene, which is regulated by a constitutive promoter, to normalise unspecific effects linked to cell number or serum matrix effect. Human TNF and TNF-inhibitors prior incubated with the cytokine was added to the cell line; since no other cytokines signalling through NFκB were able to activate the reporter gene, the response observed was specific for TNF. The reported-gene assay is able to quantify both residual anti-TNF drug activity and anti-drug neutralising antibodies by demonstrating increase or reduction of FL/RL expression (62).

Human MAb treatment can cause the formation of anti-allotype antibodies in patients lacking that allotype (67). Recently, an association has been found between patient allotype and anti-IFX antibody concentration (67). Similar to IFX (68), ADA expresses the G1m1,17-allotype (69). Bartelds *et al*. used RIA and a two-sided bridging ELISA to investigate the immunogenicity of the ADA allotype, but failed to confirm the hypothesis that a mismatch between ADA allotype and the allotypes of the IgG of ADA-treated RA patients is associated with a higher anti-ADA frequency (69). A possible interference of RF in anti-drug detection has been hypothesised, but little influence of RF on anti-IFX ELISA or RIA tests has been detected (8, 14, 17).

Influence of anti-drug antibodies on clinical response

The response to TNF antagonists parallels the drug trough levels. As long as the anti-drug antibody level is lower than drug trough level, treatment can be effective; when antibody production exceeds serum drug levels, the therapy becomes ineffective and free anti-drug antibodies can be detected (3). To our knowledge, mature and relevant data on neutralising antibodies are available only for IFX and ADA, whereas neutralising antibodies have never been reported with ETA. Data on CZP and GLM have been collected only in registrative trials, and definite conclusions on the potential reduction of clinical response following the development of antibodies against these molecules cannot yet be drawn.

Infliximab

Repeated infusions of IFX, increased doses or more frequent drug administration may be required because of loss of response and (in almost 50% of patients) discontinuation due to infusion-related adverse events may occur. Enhanced drug clearance due to immune complex formation could represent a mechanism by which anti-IFX antibodies mediate a reduction in clinical efficacy.

A study conducted by van der Laken *et al*. investigated the mechanism of non-response to anti-TNF treatment by using radiolabelled drug (70). Two responding and two non-responding RA patients were infused with ^{99m}Tc-IFX. In both non-responders, all infused IFX was bound to circulating anti-IFX antibodies within the first 30 minutes of infusion, resulting in low levels of drug. At 30–60 min of infusion, the concentration of IFX dominated that of anti-IFX antibody and drug levels increased. Imaging suggested a faster clearance and higher liver and spleen uptake of IFX in non-responders, possibly due to removal of immune complexes by the macrophages-phagocytes system (70).

Clinical studies have confirmed that anti-IFX antibody levels inversely correlate with trough IFX levels and clinical response (7, 8, 11, 17), and anti-drug antibodies have been detected in up to 100% of non-responders (9). Two studies found that 42–47% of patients with acquired drug resistance, requiring higher doses of IFX or shorter infusion interval, had high circulating anti-IFX and low post-infusion IFX concentrations (6, 10). Moreover, the kinetics of anti-drug emergence could influence long-term clinical outcome; in a cohort of 106 RA patients, early formation (within 3 months) of anti-IFX antibodies has been associated with therapy discontinuation (8).

In AS, anti-IFX antibodies have been associated with undetectable serum

trough IFX concentration and lower response. In a study investigating the effect on clinical response in 38 SA patients, only 9% of patients with detectable anti-IFX antibodies were responders at week 54, compared with 74% of patients without antibodies (17). More recently, in a larger, long-term study in 94 SpA patients, a significantly higher disease activity at 6, 12 and >48 months has been associated with anti-drug antibody positivity (p=0.038, p=0.042 and p=0.024 *versus* anti-IFX antibody negative patients, respectively) (15).

Adalimumab

A direct effect on trough serum drug concentration and treatment efficacy has been also been described for anti-ADA antibodies. In a cohort of 272 RA patients, those with anti-ADA antibodies had significantly lower ADA concentrations compared with patients showing no immune response to the drug, regardless of the antibodies titre (26); moreover, after 3 years of follow-up, patients with anti-ADA positivity have had a significantly worst clinical response compared to those who tested negative (54). Anti-ADA antibodies could be predictors of clinical response and were detected more often in non-responders compared with moderate/good responders; moreover, anti-ADA positive patients showed lower improvement in DAS28 and less often achieved sustained low disease activity or remission (9, 22, 26). In a long-term study, drop out for treatment failure was recorded more often in patients who tested positive for anti-ADA antibodies (26). In poorly-responding patients, the increase in dosing frequency often resulted in the disappearance of anti-ADA and in an increase of ADA serum concentration, either as a result of immunotolerance or due to excess drug (22, 26). However, the clinical benefit of ADA dose escalation is debatable: approximately 80% of non-responsive patients did not achieve minimal disease activity after increased dosing even when anti-ADA become undetectable (26).

Other anti-TNF

Among the various studies investigating immunogenicity of ETA in rheumatic diseases (13, 32, 34, 35), only Dore *et al*. detected anti-ETA antibodies in 5.6% of 222 RA patients during a 24-week observation period (32); since all the antibodies were non-neutralising (as detected by neutralising antibody ELISA), no effect on drug efficacy was observed (32). Data on whether anti-drug antibodies to CZP and GLM might affect the clinical response are too scant to conclude. One study has shown that the ACR20 response rate at week 24 was reduced by approximately 5% in patients positive for anti-CZP antibody (38). Differences in clinical response among patients with and without anti-GLM were not significant, although a higher percentage of ACR20 and ACR50 responders belonged to the anti-GLM negative group (45).

Effects on switching to a second anti-TNF

Clinical response to a second TNF inhibitor is similar in patients who developed anti-drug antibodies to the first anti-TNF and in patients who are TNF naïve (25).

Among a population of 235 RA patients treated with ADA, anti-ADA antibodies were detected in 20% of patients and were associated with a lower DAS28 score improvement (25). Moreover, 52 patients received IFX prior to ADA, and among switchers, 33 (63%) tested positive for anti-IFX at ADA baseline and formed anti-ADA antibodies more often compared with TNF naïve patients. After 28 weeks, clinical response to ADA was significantly greater in TNF naïve and slightly higher in switched patients with anti-IFX antibodies than in anti-IFX negative switched patients; the response to ADA in the two groups of switched patients was not significantly influenced by the previous development of anti-IFX antibodies (25). In another study, a clinically meaningful improvement in all effectiveness endpoints was observed after 16 weeks of exposure to ADA, regardless of the anti-IFX status at baseline (47% anti-IFX positive *vs*. 43% negative); anti-ADA antibodies were detected in 2 (out of 41) patients, both of whom were also anti-IFX positive (27).

The effect of anti-IFX and anti-ADA antibodies on the efficacy of ETA has been tested in a cohort of 292 patients (71). Among these, 30 patients were previously treated with IFX and 59 with ADA; anti-IFX and anti-ADA, assessed at baseline by RIA at time of switch to ETA, were positive in 53% of patients (71). After 28 weeks of ETA treatment, patients who switched from a previous anti-TNF achieved a clinical response comparable to TNF naïve patients. As previously described (25), the immunogenicity of the first anti-TNF did not affect the efficacy of the subsequent drug since switchers who were anti-IFX or anti-ADA negative showed a lower response to ETA compared to those patients who were anti-drug antibody positive or TNF naïve (71). Therefore, different mechanisms may account for different type of non-response to a second TNF antagonist.

Influence of anti-drug antibodies on drug safety

Patients who develop anti-drug antibodies are more likely to experience infusion-related reactions (3) and other potential adverse events, such as thromboembolic events (72). To our knowledge, to date such events have only been associated with IFX and ADA.

Infliximab

Several types of acute infusion reactions have been described during IFX treatment (Table VI). Data from RA patients demonstrated that infusion reactions were associated with anti-IFX positivity (7, 11) and early anti-drug detection seems to be associated to discontinuation of therapy for acute reactions, but not for other adverse events. In the study by van der Laken *et al*., the single patient with severe infusion reaction showed IFX-anti-IFX complexes of various sizes, including ones weighing >1.000.000 Da) (70). Simi-

Table VI. Acute local and systemic reactions during infliximab infusions.

Glottal/labial/facial oedema
Urticaria
Chest pain, dyspnoea
Cough/tickling throat
Hypotension, shock

larly, in a study in AS patients, infusion reactions occurred only in patients who were anti-IFX positive (17). In another study, conducted in 108 rheumatic patients treated with IFX, infusion reactions were observed in 52% of anti-IFX positive and 1% of anti-IFX negative patients (12). Finally, in a recent study conducted by Vultaggio *et al*., infusion reactions were observed in 11 out of 71 IFX treated patients (15.4%): serum anti-IFX were detected in a significantly higher percentage of patients who experienced an infusion reactions ("reactive") compared to "unreactive" patients (72.7% *vs*. 18.1%, $p<0.03$) (73). Among "reactive" patients, 3/11 (27.2%) tested positive for anti-IFX IgE and developed a positive skin test (at least 3 mm wheal area) 15 minutes after the intradermal inoculation of 1:10 diluted IFX (73). Conversely, in another study, among 315 patients exposed to IFX, 8% developed infusion reactions which were strongly associated with an IgG, but not with an IgE response to IFX (74).

Adalimumab

In a cohort study, anti-ADA antibodies have been implicated in increased thrombotic risk (72): among 272 patients, 8 cases of thromboembolic events were reported and the incidence rate was higher in those with detectable anti-ADA antibodies than those without (HR 3.8 [95% confidence interval 0.9–15.3]; $p=0.064$). The formation of immune complexes might be implicated in the occurrence of venous thromboembolism due to their ability to induce a prothrombotic state: immune complexes can activate platelets via the Fcγ receptor and complement system, and stimulate aggregation and procoagulant microparticle release (72). Alternatively, anti-ADA emergence could represent an epiphenomenon associated to the inflammation status related to the thrombotic episode.

Conclusions and implications for clinical practice

Before concluding, we should point out that these views and conclusions are drawn from a narrative review, which is subject to the usual limitations due to the non-systematic nature of the selection of studies and data included were not subject to any formal statistical analysis. However, heterogeneity of the reported data and differences in methodological approach to immunogenicity of anti-TNF drugs make a systematic review on this topic difficult.

All available anti-TNF drugs may induce an immune response. However, the variation in incidence of anti-drug antibodies, as well as the impact on efficacy and safety, can be explained by drug conformation, use of concomitant immunosuppressant, dosing regimen and route of administration. Data on new anti-TNFs (*e.g*. CZP and GLM) are still scant, and their immunogenic potential needs to be ascertained from large surveillance studies and clinical experience. An association between anti-drug antibodies and low serum drug concentrations is clinically relevant, since it is likely related to a low response. In this regard, knowledge of the relevance of non-neutralising *versus* neutralising anti-drug antibodies needs to be extended, since the latter can actually affect clinical response; however, other mechanisms, such as modified clearance of drug bound within immune complexes, might also be responsible for reduction of drug levels and loss of response. Strict monitoring of both trough drug levels and anti-drug antibodies might be useful for tailoring therapy in individuals and immunogenicity assessment is needed in order to identify patients who will benefit from a change in anti-TNF therapy. Patients who failed their first-course TNF inhibitor for reasons other than an immunogenic response to the drug will probably gain more from switching to a treatment with a different mechanism of action. Conversely, TNF inhibitor immunogenicity does not affect the effectiveness of a different anti-TNF agent in patients who develop anti-drug antibodies specific to the first anti-TNF; therefore, switching to a drug of the same class might be effective. Further investigation in well-designed clinical trials is required to optimise the identification and selection of patients who may benefit from switching to another anti-TNF.

To date, it is difficult to compare the immunogenicity of the different TNF inhibitors; the huge variability of results due to methodological heterogeneity in the approach if this issue precludes the comparison between different molecules. The actual impact of immunogenicity will be fully realised when drug and anti-drug antibodies levels will be monitored routinely. In order to optimise effectiveness and safety of therapeutic proteins, careful monitoring of their bioavailability and immunogenicity is desirable; however, in order to routinely monitoring anti-drug antibodies, further investigation of accurate and cost-effective methods of antibody detection is needed.

References

1. TAYLOR PC: Pharmacology of TNF blockade in rheumatoid arthritis and other chronic inflammatory diseases. *Curr Opin Pharmacol* 2010; 10: 308-15.
2. SCOTT DL: Biologics-based therapy for the treatment of rheumatoid arthritis. *Clin Pharmacol Ther* 2012; 91: 30-43.
3. WOLBINK GJ, AARDEN LA, DIJKMANS BA: Dealing with immunogenicity of biologicals: assessment and clinical relevance. *Curr Opin Rheumatol* 2009; 21: 211-5.
4. VERONESE FM, MERO A: The impact of PEGylation on biological therapies. *BioDrugs* 2008; 22: 315-29.
5. MAINI RN, BREEDVELD FC, KALDEN JR *et al*.: Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 1552-63.
6. HARAOUI B, CAMERON L, OUELLET M, WHITE B: Anti-infliximab antibodies in patients with rheumatoid arthritis who require higher doses of infliximab to achieve or maintain a clinical response. *J Rheumatol* 2006; 33: 31-6.
7. WOLBINK GJ, VIS M, LEMS W *et al*.: Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 711-5.
8. 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* 2006; 54: 3782-9.
9. RADSTAKE TR, SVENSON M, EIJSBOUTS AM *et al*.: Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 2009; 68: 1739-45.
10. FINCKH A, DUDLER J, WERMELINGER F *et al*.: Influence of anti-infliximab antibodies

and residual infliximab concentrations on the occurrence of acquired drug resistance to infliximab in rheumatoid arthritis patients. *Joint Bone Spine* 2010; 77: 313-8.
11. PASCUAL-SALCEDO D, PLASENCIA C, RAMIRO S *et al*.: Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis. *Rheumatology* (Oxford) 2011; 50: 1445-52.
12. DUCOURAU E, MULLEMAN D, PAINTAUD G *et al*.: Antibodies toward infliximab are associated with low infliximab concentration at treatment initiation and poor infliximab maintenance in rheumatic diseases. *Arthritis Res Ther* 2011; 13: R105.
13. HOSHINO M, YOSHIO T, ONISHI S, MINOTA S: Influence of antibodies against infliximab and etanercept on the treatment effectiveness of these agents in Japanese patients with rheumatoid arthritis. *Mod Rheumatol* 2012; 22: 532-40.
14. VAN DEN BEMT BJ, DEN BROEDER AA, WOLBINK GJ *et al*.: Anti-infliximab antibodies are already detectable in most patients with rheumatoid arthritis halfway through an infusion cycle: an open-label pharmacokinetic cohort study. *BMC Musculoskelet Disord* 2011; 12: 12.
15. PLASENCIA C, PASCUAL-SALCEDO D, NUÑO L *et al*.: Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab. *Ann Rheum Dis* 2012; 71: 1955-60.
16. KAVANAUGH A, KRUEGER GG, BEUTLER A *et al*.: Infliximab maintains a high degree of clinical response in patients with active psoriatic arthritis through 1 year of treatment: results from the IMPACT 2 trial. *Ann Rheum Dis* 2007; 66: 498-505.
17. DE VRIES MK, WOLBINK GJ, STAPEL SO *et al*.: Decreased clinical response to infliximab in ankylosing spondylitis is correlated with anti-infliximab formation. *Ann Rheum Dis* 2007; 66: 1252-4.
18. KOSMAČ M, AVČIN T, TOPLAK N, SIMONINI G, CIMAZ R,CURIN ŠERBEC V: Exploring the binding sites of anti-infliximab antibodies in pediatric patients with rheumatic diseases treated with infliximab. *Pediatr Res* 2011; 69: 243-8.
19. WEINBLATT ME, KEYSTONE EC, FURST DE *et al*.: Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum* 2003; 48: 35-45.
20. KEYSTONE EC, KAVANAUGH AF, SHARP JT *et al*.: Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum* 2004; 50: 1400-11.
21. VAN DE PUTTE LB, ATKINS C, MALAISE M *et al*.: Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann Rheum Dis* 2004; 63: 508-16.
22. BARTELDS GM, WIJBRANDTS CA, NURMOHAMED MT *et al*.: Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 2007; 66: 921-6.
23. BENDER NK, HEILIG CE, DROLL B, WOHLGEMUTH J, ARMBRUSTER FP, HEILIG B: Immunogenicity, efficacy and adverse events of adalimumab in RA patients. *Rheumatol Int* 2007; 27: 269-74.
24. VAN SCHOUWENBURG PA, BARTELDS GM, HART MH, AARDEN L, WOLBINK GJ, WOUTERS D: A novel method for the detection of antibodies to adalimumab in the presence of drug reveals "hidden" immunogenicity in rheumatoid arthritis patients. *J Immunol Methods* 2010; 362: 82-8.
25. BARTELDS GM, WIJBRANDTS CA, NURMOHAMED MT *et al*.: Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. *Ann Rheum Dis* 2010; 69: 817-21.
26. BARTELDS GM, KRIECKAERT CL, NURMOHAMED MT *et al*.: Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *Jama* 2011; 305: 1460-8.
27. VAN DER BIJL AE, BREEDVELD FC, ANTONI CE *et al*.: An open-label pilot study of the effectiveness of adalimumab in patients with rheumatoid arthritis and previous infliximab treatment: relationship to reasons for failure and anti-infliximab antibody status. *Clin Rheumatol* 2008; 27: 1021-8.
28. DE VRIES MK, BROUWER E, VAN DER HORST-BRUINSMA IE *et al*.: Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. *Ann Rheum Dis* 2009; 68: 1787-8.
29. VAN KUIJK AW, DE GROOT M, STAPEL SO, DIJKMANS BA, WOLBINK GJ, TAK PP: Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. *Ann Rheum Dis* 2010; 69: 624-5.
30. IMAGAWA T, TAKEI S, UMEBAYASHI H *et al*.: Efficacy, pharmacokinetics, and safety of adalimumab in pediatric patients with juvenile idiopathic arthritis in Japan. *Clin Rheumatol* 2012; 31: 1713-21.
31. DORE RK, MATHEWS S, SCHECHTMAN J *et al*.: The immunogenicity, safety, and efficacy of etanercept liquid administered once weekly in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2007; 25: 40-6.
32. KEYSTONE EC, SCHIFF MH, KREMER JM *et al*.: Once-weekly administration of 50 mg etanercept in patients with active rheumatoid arthritis: results of a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2004; 50: 353-63.
33. KLARESKOG L, GAUBITZ M, RODRIGUEZ-VALVERDE V, MALAISE M, DOUGADOS M, WAJDULA J: Assessment of long-term safety and efficacy of etanercept in a 5-year extension study in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2011; 29: 238-47.
34. JAMNITSKI A, KRIECKAERT CL, NURMOHAMED MT *et al*.: Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. *Ann Rheum Dis* 2012; 71: 88-91.
35. DE VRIES MK, VAN DER HORST-BRUINSMA IE, NURMOHAMED MT *et al*.: Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009; 68: 531-5.
36. MEASE PJ, KIVITZ AJ, BURCH FX *et al*.: Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. *Arthritis Rheum* 2004; 50: 2264-72.
37. KEYSTONE E, HEIJDE DV, MASON D JR *et al*.: Certolizumab pegol plus methotrexate is significantly more effective than placebo plus methotrexate in active rheumatoid arthritis: findings of a fifty-two-week, phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Arthritis Rheum* 2008; 58: 3319-29.
38. FLEISCHMANN R, VENCOVSKY J, VAN VOLLENHOVEN RF *et al*.: Efficacy and safety of certolizumab pegol monotherapy every 4 weeks in patients with rheumatoid arthritis failing previous disease-modifying antirheumatic therapy: the FAST4WARD study. *Ann Rheum Dis* 2009; 68: 805-11.
39. SMOLEN J, LANDEWÉ RB, MEASE P *et al*.: Efficacy and safety of certolizumab pegol plus methotrexate in active rheumatoid arthritis: the RAPID 2 study. A randomised controlled trial. *Ann Rheum Dis* 2009; 68: 797-804.
40. CHOY E, MCKENNA F, VENCOVSKY J *et al*.: Certolizumab pegol plus MTX administered every 4 weeks is effective in patients with RA who are partial responders to MTX. *Rheumatology* (Oxford) 2012; 51: 1226-34.
41. KAY J, MATTESON EL, DASGUPTA B *et al*.: Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate: a randomized, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum* 2008; 58: 964-75.
42. EMERY P, FLEISCHMANN RM, MORELAND LW *et al*.: 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* 2009; 60: 2272-83.
43. KEYSTONE EC, GENOVESE MC, KLARESKOG L *et al*.: Golimumab, a human antibody to tumour necrosis factor {alpha} given by monthly subcutaneous injections, in active rheumatoid arthritis despite methotrexate therapy: the GO-FORWARD Study. *Ann Rheum Dis* 2009; 68: 789-96.
44. SMOLEN JS, KAY J, DOYLE MK *et al*.: Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor alpha inhibitors (GO-AFTER study): a multicentre, randomised, double-blind, placebo-controlled, phase III trial. *Lancet* 2009; 374: 210-21.
45. KREMER J, RITCHLIN C, MENDELSOHN A, *et al*.: Golimumab, a new human anti-tumor

necrosis factor alpha antibody, administered intravenously in patients with active rheumatoid arthritis: Forty-eight-week efficacy and safety results of a phase III randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2010; 62: 917-28.
46. TANAKA Y, HARIGAI M, TAKEUCHI T *et al*.: Golimumab in combination with methotrexate in Japanese patients with active rheumatoid arthritis: results of the GO-FORTH study. *Ann Rheum Dis* 2012; 71: 817-24.
47. WEINBLATT ME, BINGHAM CO, 3RD, MENDELSOHN AM *et al*.: Intravenous golimumab is effective in patients with active rheumatoid arthritis despite methotrexate therapy with responses as early as week 2: results of the phase 3, randomised, multicentre, double-blind, placebo-controlled GO-FURTHER trial. *Ann Rheum Dis* 2012:
48. ZHUANG Y, XU Z, FREDERICK B *et al*.: Golimumab pharmacokinetics after repeated subcutaneous and intravenous administrations in patients with rheumatoid arthritis and the effect of concomitant methotrexate: an open-label, randomized study. *Clin Ther* 2012; 34: 77-90.
49. KAVANAUGH A, MCINNES I, MEASE P *et al*.: Golimumab, a new human tumor necrosis factor alpha antibody, administered every four weeks as a subcutaneous injection in psoriatic arthritis: Twenty-four-week efficacy and safety results of a randomized, placebo-controlled study. *Arthritis Rheum* 2009; 60: 976-86.
50. KAVANAUGH A, VAN DER HEIJDE D, MCINNES IB *et al*.: Golimumab in psoriatic arthritis: one-year clinical efficacy, radiographic, and safety results from a phase III, randomized, placebo-controlled trial. *Arthritis Rheum* 2012; 64: 2504-17.
51. INMAN RD, DAVIS JC, JR., HEIJDE D *et al*.: Efficacy and safety of golimumab in patients with ankylosing spondylitis: results of a randomized, double-blind, placebo-controlled, phase III trial. *Arthritis Rheum* 2008; 58: 3402-12.
52. BRAUN J, BARALIAKOS X, HERMANN KG *et al*.: Golimumab reduces spinal inflammation in ankylosing spondylitis: MRI results of the randomised, placebo- controlled GO-RAISE study. *Ann Rheum Dis* 2012; 71: 878-84.
53. VAN DER MAAS A, VAN DEN BEMT BJ, WOLBINK G, 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* 2012; 13: 184.
54. KRIECKAERT CL, JAMNITSKI A, NURMOHAMED MT, KOSTENSE PJ, BOERS M,WOLBINK G: Comparison of long-term clinical outcome with etanercept treatment and adalimumab treatment of rheumatoid arthritis with respect to immunogenicity. *Arthritis Rheum* 2012; 64: 3850-5.
55. MAYUMI H, GOOD RA: Long-lasting skin allograft tolerance in adult mice induced across fully allogeneic (multimajor H-2 plus multiminor histocompatibility) antigen barriers by a tolerance-inducing method using cyclophosphamide. *J Exp Med* 1989; 169: 213-38.
56. MAYUMI H, HIMENO K, SHIN T, NOMOTO K: Drug-induced tolerance to allografts in mice. VI. Tolerance induction in H-2-haplotype-identical strain combinations in mice. *Transplantation* 1985; 40: 188-94.
57. BENDTZEN K: Is there a need for immunopharmacologic guidance of anti-tumor necrosis factor therapies? *Arthritis Rheum* 2011; 63: 867-70.
58. CASSINOTTI A, TRAVIS S: Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: a critical systematic review. *Inflamm Bowel Dis* 2009; 15: 1264-75.
59. WOLBINK GJ, VOSKUYL AE, LEMS WF *et al*.: 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* 2005; 64: 704-7.
60. KALIYAPERUMAL A, JING S: Immunogenicity assessment of therapeutic proteins and peptides. *Curr Pharm Biotechnol* 2009; 10: 352-8.
61. GUDBRANDSDOTTIR S, LARSEN R, SORENSEN LK *et al*.: TNF and LT binding capacities in the plasma of arthritis patients: effect of etanercept treatment in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2004; 22: 118-24.
62. LALLEMAND C, KAVROCHORIANOU N, STEENHOLDT C *et al*.: Reporter gene assay for the quantification of the activity and neutralizing antibody response to TNF-alpha antagonists. *J Immunol Methods* 2011; 373: 229-39.
63. RISPENS T, DE VRIEZE H, DE GROOT E *et al*.: Antibodies to constant domains of therapeutic monoclonal antibodies: anti-hinge antibodies in immunogenicity testing. *J Immunol Methods* 2012; 375: 93-9.
64. AARDEN L, RUULS SR, WOLBINK G: Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement. *Curr Opin Immunol* 2008; 20: 431-5.
65. VAN SCHOUWENBURG PA, VAN DE STADT LA, DE JONG RN *et al*.: Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. *Ann Rheum Dis* 2013; 72: 104-9.
66. SVENSON M, GEBOREK P, SAXNE T, BENDTZEN K: Monitoring patients treated with anti-TNF-alpha biopharmaceuticals: assessing serum infliximab and anti-infliximab antibodies. *Rheumatology* (Oxford) 2007; 46: 1828-34.
67. MAGDELAINE-BEUZELIN C, VERMEIRE S, GOODALL M *et al*.: IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of antibodies-to-infliximab. *Pharmacogenet Genomics* 2009; 19: 383-7.
68. JEFFERIS R, LEFRANC MP: Human immunoglobulin allotypes: possible implications for immunogenicity. *MAbs* 2009; 1: 332-8.
69. BARTELDS GM, DE GROOT E, NURMOHAMED MT *et al*.: Surprising negative association between IgG1 allotype disparity and anti-adalimumab formation: a cohort study. *Arthritis Res Ther* 2010; 12: R221.
70. VAN DER LAKEN CJ, VOSKUYL AE, ROOS JC *et al*.: Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. *Ann Rheum Dis* 2007; 66: 253-6.
71. JAMNITSKI A, BARTELDS GM, NURMOHAMED MT *et al*.: The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. *Ann Rheum Dis* 2011; 70: 284-8.
72. KORSWAGEN LA, BARTELDS GM, KRIECKAERT CL *et al*.: Venous and arterial thromboembolic events in adalimumab-treated patients with antiadalimumab antibodies: a case series and cohort study. *Arthritis Rheum* 2011; 63: 877-83.
73. VULTAGGIO A, MATUCCI A, NENCINI F *et al*.: Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. *Allergy* 2010; 65: 657-61.
74. STEENHOLDT C, SVENSON M, BENDTZEN K, THOMSEN OO, BRYNSKOV J, AINSWORTH MA: Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 34: 51-8.