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Chapter 11

Summary and General discussion

This thesis comprises several studies covering pivotal aspects of the immunogenicity of monoclonal antibodies (mAbs). The main questions addressed by the present thesis are:

- Is immunogenicity clinically relevant?
 - Regarding efficacy
 - Regarding adverse events
- Which factors influence immunogenicity?
- How should clinicians deal with immunogenicity?

Immunogenicity of fully human monoclonal antibodies

The first murine-derived monoclonal antibodies had been recognized to provoke a human anti-murine antibody (HAMA) response, (1;2) that limited the clinical use of these antibodies. Efforts were made to humanize mAbs to chimeric (and fully human) antibodies. However, the immunogenicity of chimeric monoclonal antibody infliximab in RA patients had already been described 13 years ago. (3) Furthermore, immunologists have recognized that the basic idea that we are tolerant of all self proteins, and only respond to foreign proteins is flawed. (4) New clones of B-cells with unique idiotypes are generated every day throughout life by the processes of somatic gene recombination and somatic mutation. It is unlikely that tolerance to each B-cell clone is generated as soon as every new sequence emerges. (4) It seems obvious that anti-idiotypic immune responses are directed against fully human mAbs as well, i.e. in the form of human anti-human antibody responses (HAHA). Despite the rapid progress in mAb development in the last decades, knowledge on the immunogenicity of mAbs is only just emerging. This can be explained by the challenges involved in investigating immunogenicity. For instance, technical and standardization issues, such as the lack of clarity regarding the development of valid assays and of comparability of results between studies, are difficulties. In addition, power problems are likely because anti-drug antibodies develop only in part of the mAb treated patients. At the beginning of the mAb era, sufficient study population numbers, serum samples and financial opportunities to tackle the technical aspects were only available within the pharmaceutical industry.

Before the publication of the first article of this thesis, **Chapter 1**, data on anti-drug antibody formation against fully human monoclonal antibodies was limited. Only a few data on anti-drug antibody formation against murine and chimeric monoclonal antibodies was available, and some reports describing negative effects on treatment response had been published. (3;5;6) Such relationships had not yet been described for adalimumab, a fully human monoclonal antibody. HAHA were detected in 12 percent of all study patients in an investigation where patients were treated with adalimumab monotherapy (i.e. without concomitant methotrexate). (7) However, no statistically significant difference in ACR 20 response between the groups with and without antibodies to adalimumab had been found. At that time the question was raised whether antibodies against fully human monoclonal antibodies were clinically relevant or not. The first evidence for the clinical relevance of HAHA was given in **Chapter 1**, which demonstrated in a case report that the formation of HAHA to adalimumab could have been an explanation for failure of adalimumab therapy.

Chapter 2 showed that in a population of 121 adalimumab-treated rheumatoid arthritis patients anti-adalimumab antibodies developed in 21 patients (17%) during the first 28 weeks of treatment. The presence of anti-drug antibodies was associated with low to undetectable serum adalimumab levels and less clinical improvement. The antibodies against adalimumab are directed against the idotype of adalimumab and are therefore neutralizing. Furthermore, the low to undetectable serum adalimumab

concentrations might be due to the increased clearance of the immune complexes (adalimumab- anti-adalimumab antibody) by the liver as was observed in a study with ⁹⁹technetium-labeled infliximab. (8) Subsequent to the studies described above, anti-adalimumab antibodies have been described in several diseases for which adalimumab treatment is indicated. The incidence of anti-adalimumab antibodies varies in these studies from 2,6%-45%. (7;9-19)

The effect of anti-drug antibodies on treatment response is clinically relevant

The majority of the aforementioned studies showed that anti-adalimumab antibodies were associated with diminished serum adalimumab concentrations and a diminished treatment response (9;11;12;14;17-19) and therefore correspond with our findings. Long-term data on immunogenicity are scarce. In Crohn's disease one study described the long-term outcome of adalimumab treatment with a focus on immunogenicity. (12) Data showed that adalimumab trough serum concentration was lower during the entire follow-up period (median 20 months) in patients who discontinued therapy and was affected by the presence of antibodies against adalimumab. In patients who displayed an adalimumab trough concentration <0.33 µg/ml at least once, sustained clinical benefit decreased in comparison to patients never having low trough serum concentrations. However, these results should be interpreted with caution due to the limited number of patients, as also argued by the authors, therefore no definite conclusions about the long term association between anti-drug antibodies and sustained decreased clinical benefit could be drawn.

In **Chapter 3** our aim was to investigate the course of anti-drug antibody development and its clinical relevance as measured by effects on treatment discontinuation, disease activity, and remission during long-term (3 year) follow-up. Therefore, we followed a cohort of 272 consecutive rheumatoid arthritis patients treated with adalimumab, and measured anti-adalimumab antibody (AAA) development to investigate the described outcome measures. During three years 76 out of 272 patients (28%) developed antibodies against adalimumab (AAA), of whom 51 (67%) developed AAA during the first 28 weeks of treatment. The results of this study showed that the development of anti-drug antibodies has a large and clinically relevant negative impact on the treatment-effect of adalimumab in rheumatoid arthritis patients. Not only did patients with AAA discontinue treatment more often and earlier than patients without AAA, they also had a higher disease activity during treatment and only rarely went into remission. In conclusion, the development of anti-drug antibodies jeopardizes the long-term efficacy of adalimumab treatment in rheumatoid arthritis patients in clinical practice; the long-term treatment goals rheumatologists desire for their patients are not achieved.

Assessment of immunogenicity helps in rational clinical decision making

The lack of response to mAbs can partly be explained by an immunogenic response against these drugs, but there are also non-responding patients in whom an immunogenic reaction cannot be demonstrated. (5) Lack of response to TNF blockade in these patients might be ascribed to mechanisms that are not primarily driven by TNF. (20;21) Hence, there seem to be different types of non-responders with different underlying pathogenic mechanisms causing non-response. Knowing the reason for non-response would be helpful in determining whether patients who fail one TNF blocker should switch to another TNF antagonist or to a drug with a different mechanism of action. Therefore, we investigated immunogenicity in respect to switching TNF inhibitors in a population of 235 rheumatoid arthritis patients. **Chapter 4** demonstrates that anti-TNF naive patients had a better response to adalimumab therapy than prior infliximab non-responders. These infliximab non-responders were divided into patients with and without anti-infliximab antibodies in order to estimate whether the non-response to the first TNF-inhibitor infliximab was attributable to an immunogenic

reaction or to another reason. Prior infliximab non-responders without anti-infliximab antibodies had the least improvement with subsequent adalimumab therapy. Previous studies on switching biologicals also identified different patient groups based on their response after switching; primary failures (patients with no response/intolerance, unlike secondary failures/patients with loss of response) to previous infliximab had a poor response to subsequent adalimumab therapy. (10;22;23) As stated before: there may be a subpopulation of RA patients that does not respond to anti-TNF therapy. (22;24) Further evidence for the latter is given by a study that showed that high levels of circulating TNF bioactivity was associated with a good clinical response to infliximab. (21) TNF may not be the crucial cytokine instigating rheumatoid arthritis in primary non-responders to anti-TNF therapy. Another study showed that responders to infliximab had a significantly higher synovial TNF expression and significantly more infiltration by TNF producing inflammatory cells than non-responders. (20) Therefore, our study suggests that non-responders to TNF blockers should be treated differently depending on their anti-drug antibody status. Antibody-positive patients probably benefit most from switching to a less immunogenic drug acting on the same principle, or from optimizing concomitant DMARD (MTX) therapy. Furthermore, it is likely that in non-responders without anti-TNF blocking antibodies it is more useful and cost-effective to start treatment based on a mechanism of action other than TNF blockade.

Proof for this concept has been gained by the follow-up study described in **Chapter 5**. This cohort study comprised 292 consecutive RA patients, all treated with etanercept. Eighty-nine patients (30%) were treated previously with infliximab or adalimumab ("switchers"), and the remaining 203 (70%) were anti-TNF naive. All switchers were divided into two groups: with and without antibodies against the previous biological. After 28 weeks of therapy response to etanercept did not differ between anti-TNF naive patients and switchers with anti-drug antibodies. In contrast, switchers without anti-drug antibodies had a diminished response to etanercept treatment compared to both TNF naive patients and switchers with antibodies. In conclusion, determining the immunogenic status of a non-responding patient should be part of a personalized treatment regimen. It can help to decide in which patient switching to another TNF inhibitor might be beneficial and appropriate considering the high costs of anti-TNF therapy.

Patient and treatment related factors associated with immunogenicity

Another important new finding from **Chapter 4** was that patients who previously formed antibodies against infliximab were more likely to develop antibodies against adalimumab. A possible explanation why people develop antibodies against both drugs might be that some patients are more susceptible to develop an immune response, probably related to their genetic background.

Previous studies showed that polymorphisms in the promoter region of the interleukin 10 (IL-10) gene, a cytokine with a key role in antibody formation, are associated with the formation of inhibitory antibodies to recombinant factor VIII in hemophilia (25;26), and with the development of auto-antibodies against nicotinic acetylcholine receptor (nAChR) in myasthenia gravis. (27) In **Chapter 6** we hypothesized that polymorphisms in this gene were also associated with the formation of antibodies against anti-TNF agents. To test this hypothesis anti-adalimumab antibodies were measured 28 weeks after initiation of treatment with adalimumab in 192 prospectively followed Caucasian rheumatoid arthritis patients. We observed that distinct IL10 polymorphisms were associated with formation of antibodies against adalimumab.

Another genetic patient characteristic that theoretically could have a role in the patient's immune response against mAbs is the allotypic phenotype of a patient. Immunoglobulins carry allotypes which

represent slight differences in the amino acid sequences of the constant chains of an IgG molecule. Allotypic markers can differ between individuals and therefore immunoglobulins with certain allotypes can be immunogenic when injected into individuals whose immunoglobulins lack the allotype. Treatment with monoclonal antibodies with a certain allotype can lead to the formation of anti-allotype antibodies. In **Chapter 7** we investigated whether a mismatch in IgG allotypes between adalimumab and IgG in 250 adalimumab treated patients is associated with the development of antibodies against adalimumab. Data showed that an allotype mismatch between adalimumab and IgG in adalimumab treated patients did not lead to a higher frequency of AAA. In contrast, patients who carried the same IgG allotype as present on the adalimumab IgG molecule, had a higher frequency of anti-adalimumab antibodies compared to patients whose IgG allotype differed from adalimumab. This suggests that the allotype of adalimumab may not be (highly) immunogenic. Interestingly, our data show that anti-adalimumab antibody formation occurred more often in rheumatoid arthritis patients with the G1m17-allotype than in rheumatoid arthritis patients without this allotype, which contributes to the idea that genetic factors have a role in anti-drug antibody responses.

In addition to genetic factors, environmental factors, such as concomitant immunomodulator treatment, affect the development of anti-drug antibodies. In **Chapter 8** literature on the effect of immunomodulators, such as methotrexate and azathioprine, on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies is reviewed. We observed a generally favorable effect of immunosuppressive co-treatment on reducing the immunogenicity of therapeutic monoclonal antibodies, including the two only prospective studies that had been undertaken regarding this subject. (3;28) This corresponds to the findings described in **Chapter 2,3 and 4** which showed that patients who later developed AAA less often had received concomitant methotrexate in a lower dose, and more often had received no concomitant DMARD at all. The mechanism behind the effect of immunosuppressants on immunogenicity is not elucidated. We hypothesize that by combining immunomodulators with TmAbs, the immune response is suppressed leading to a decrease in antibody formation. In other words, methotrexate or azathioprine could block the expansion of the immune reactive cells whereby the formation of anti-TmAb antibodies is reduced. The quantity of the immune response could be more relevant than just the development of the immune response. Optimization of treatment response should be the main goal when prescribing costly biological therapeutics. Especially in those inflammatory diseases in which it is not common to prescribe concomitant immunomodulating therapy, great benefits in lowering the incidence of anti-TmAb antibodies could be achieved by the use of concomitant immunosuppressants, resulting in an increased portion of patients with therapeutic concentrations of TmAbs in their blood. The concomitant use of immunosuppressants has not been associated with a higher incidence of (serious) adverse events (29;30), however, to minimize the risk of toxicity/intolerance the minimal sufficient dose of immunosuppressants to decrease the immunogenicity of TmAbs should be assessed. Evidence based consensus on prescribing concomitant immunomodulators is urgently needed.

Adverse events associated with immunogenicity

An immune response against a drug can lead to life-threatening adverse events, such as the induction of aplastic anemia caused by anti-epoetin antibodies. (31) The presence of anti-drug antibodies against TNF-inhibitors in rheumatoid arthritis has been associated with adverse events as well. The presence of antibodies to infliximab and of immune complexes of various sizes are associated with infusion-related hypersensitivity reactions. (5;6;8;32;33) In one study higher concentrations of antibodies to infliximab predicted a higher risk of infusion reactions. (6) To date, the presence of anti-

drug antibodies is not monitored in clinical practice; therefore many patients continue mAb treatment in the presence of anti-drug antibodies, resulting in ongoing formation of immune complexes. (6;8;32) Immune complex formation will only stop if mAb treatment is discontinued. The possible hazard of the continuous stimulation of the immune system by these immune complexes has hardly been investigated. The increased immune complex formation could be more harmful than generally conceived and an increased awareness might lead to the discovery of additional associated side effects. In **Chapter 9** we first describe three patients who developed severe venous and arterial thromboembolic events during treatment with adalimumab, in whom anti-adalimumab antibodies were detected. The question arose whether the development of anti-adalimumab antibodies was associated with thromboembolic events during adalimumab treatment. In a cohort of 272 consecutive adalimumab treated RA patients we detected anti-adalimumab antibodies in 76 patients. During treatment eight thromboembolic events occurred, including pulmonary emboli, cerebrovascular accidents, myocardial infarctions, deep vein thrombosis, transient ischemic attack and optical vein thrombosis. Four of these events occurred in patients with anti-adalimumab antibodies and four in patients without anti-adalimumab antibodies. After adjustment for confounding factors we observed an increase in the risk of thromboembolic events during treatment with adalimumab for patients with anti-adalimumab compared to patients without anti-adalimumab; with a hazard ratio of 7.6. However, patient numbers were relatively small, therefore validation in other cohorts is mandatory. Nevertheless, this could be an alarm signal that there might be an increased risk for thromboembolic events in patients with an immunogenic reaction against biologicals.

Assessment of immunogenicity incorporated into a clinical model

Taking together the evidence described by the studies in this thesis, we come to the following conclusions:

- Fully human monoclonal antibodies can be immunogenic
- The effect of anti-drug antibodies on treatment response is clinically relevant and jeopardizes treatment efficacy
- Immunogenicity is associated with adverse events
- Assessment of immunogenicity aids in rational clinical decision making
- The extent of an immunogenic reaction is associated with patient and treatment related factors
- Both genetic and treatment-related risk factors can lead to a higher probability of developing anti-drug antibodies

These findings should lead to clinical implications. We recommend the monitoring of serum drug levels and anti-drug antibodies during mAb treatment in all patients in daily routine practice. Instead of not knowing why a patient does not respond to a certain drug, the measurement of drug levels and anti-drug antibodies helps in understanding why an individual patient does not respond to treatment. Even in patients with low disease activity but high anti-drug antibody titres and undetectable serum drug levels it is questionable whether the low disease activity is attributable to the drug. Because in that case patients have low disease activity despite the TNF-inhibitor, because there are no detectable drug levels in the serum. Adjusting policy based on an immunogenicity assessment could lead to more (cost)-effective treatment since patients with anti-adalimumab antibodies had a higher disease activity and hardly ever achieved remission (Chapter 3). Furthermore, evidence from Chapters 4 and 5 suggests that patients who do not respond to a TNF inhibitor despite adequate serum drug levels and the absence of anti-drug antibodies, are likely to benefit more from a therapy based on another mechanism of action than from another TNF inhibitor yet again. Finally, standard monitoring of immunogenicity could help investigate possible rare adverse events associated with the development of anti-drug antibodies.

In summary, not dealing with immunogenicity appears to be a substantial burden to both patients due to impaired treatment effect and possible adverse events and possibly to society due to pharmacoeconomic aspects, which could include both health economic aspects and treatment costs. Despite the fact that (cost-effectiveness) studies are necessary to provide conclusive data, it could be useful for clinicians to consider the effects of not dealing with immunogenicity.

For some biological drugs immunogenic reactions have been investigated, but could not be demonstrated, as for the TNF-inhibitor etanercept, which is a TNF receptor Fc-fusion protein (34;35), albeit, higher serum levels of etanercept have found in patients with a EULAR good response compared to patients with a EULAR non response. (35) Some studies found low incidences of anti-etanercept antibodies, but their clinical relevance could not be demonstrated. (36;37)

Nevertheless, even during treatment with biological drugs for which immunogenic reactions have not been found, the monitoring of serum drug levels alone is beneficial, as patients with an inadequate response to treatment despite adequate serum drug levels could benefit more from treatment with a drug based on another mechanism of action than for example TNF blockade.

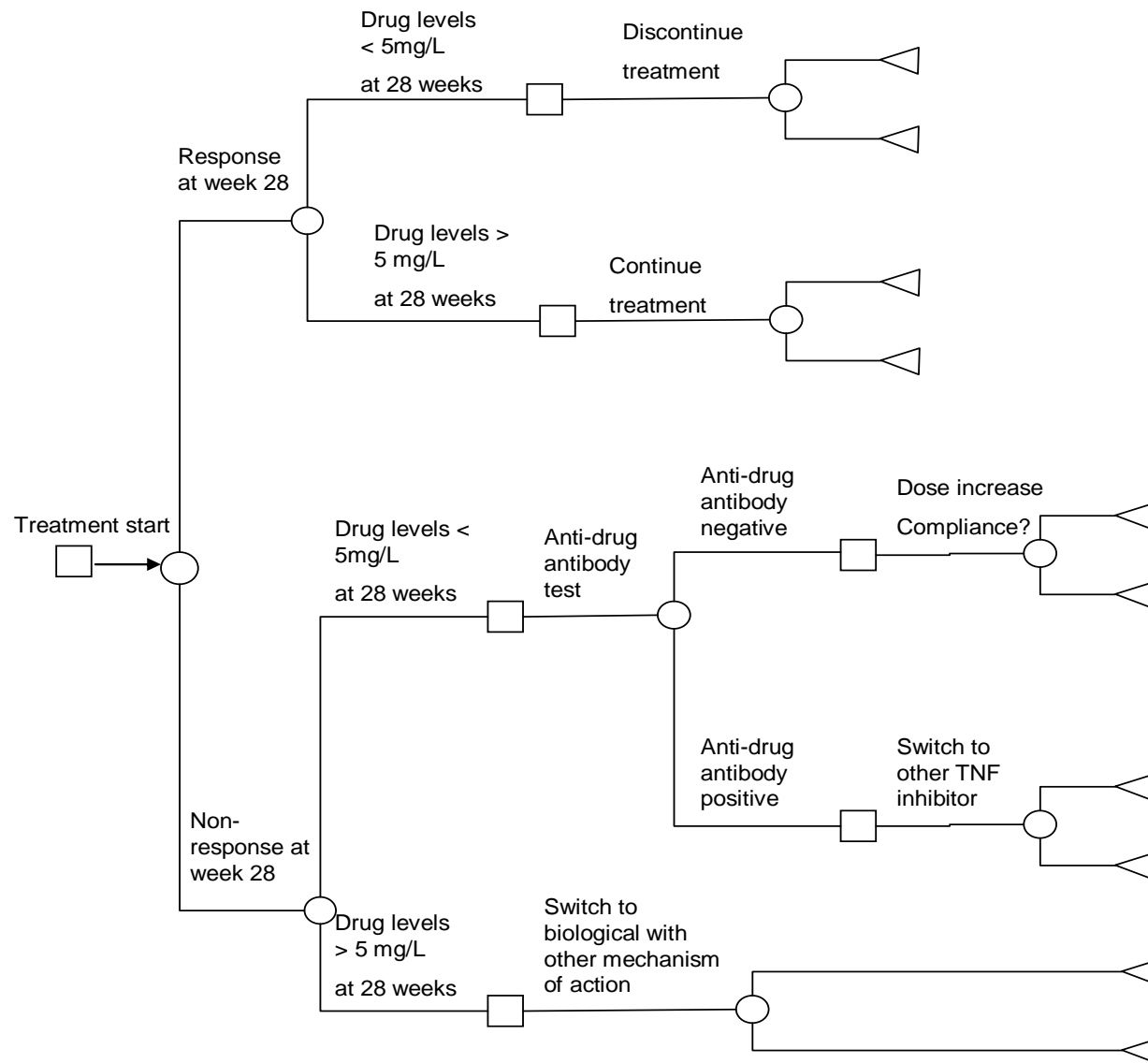


Figure 1. Incorporation of immunogenicity into a clinical model

A clinical model of how an immunogenicity assessment could be incorporated into clinical practice is shown in figure 1.

There are important practical considerations that should be taken into account when assessing an immunogenic response. First of all, reliable results can only be produced if serum samples are drawn standardized just prior to a drug injection or infusion, i.e. by trough serum sampling. In currently used assays anti-drug antibody detection is only possible if the production of anti-drug antibodies exceeds the amount of drug present in the serum due to the formation of anti-drug antibody - drug complexes. (38) Hence, drug interference will lead to an underestimation of the number of patients producing anti-drug antibodies. We have seen that antibodies against adalimumab became undetectable upon continuation of treatment (Chapter 2). This could be the result of drug interference, but it is tempting to speculate that prolonged exposure to the drug might induce tolerance. To discriminate between these two an assay which is able to detect anti-drug antibodies in the presence of drug has been developed. (39) Nevertheless, in current clinical practice and with common assays trough serum samples should be obtained to provide valid results. When in doubt, trough sampling from consecutive time points is advised.

In conclusion, we recommend drug monitoring in all patients after 28 weeks of treatment (or sooner in case of non-response) and in non-responding patients subsequently.

Future research

As described in this thesis, the field of immunogenicity research is relatively new and further research is warranted in order to acquire more knowledge about immunogenicity.

Clinical research goals in the field are:

- Development of consistent assays with appropriate specificity (and sensitivity) to support regular immunogenicity testing
- Standardization of assays
- Implementation of the clinical relevance of immunogenicity
- Development of treatment guidelines that incorporate immunogenicity testing in clinical decision making
- Prospective studies that elucidate the (beneficial) effects of immunogenicity testing incorporated into daily clinical practice
- Pharmacoeconomic studies on the effects of immunogenicity testing in daily clinical practice
- The monitoring of adverse events associated with anti-drug antibodies/ (chronic) immune complex formation
- Understanding the patient related factors influencing immunogenicity and identifying high-risk patient stratification markers
- Prospective studies to develop an evidence based consensus on prescribing concomitant immunomodulators such as methotrexate, particularly in diseases where it is not common to prescribe concomitant treatment

Reference List

- (1) Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC, Jr. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985; 45(2):879-885.
- (2) Shawler DL, Bartholomew RM, Smith LM, Dillman RO. Human immune response to multiple injections of murine monoclonal IgG. *J Immunol* 1985; 135(2):1530-1535.
- (3) Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD 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(9):1552-1563.
- (4) Clark MR. Antibody humanisation: a case of the "Emperor's new clothes"? *Immunology Today* 2000; 21:397-402.
- (5) Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT et al. Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54(3):711-715.
- (6) Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, Carbonez A et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003; 348(7):601-608.
- (7) van de Putte LB, Atkins C, Malaise M, Sany J, Russell AS, van Riel PL 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(5):508-516.
- (8) van der Laken CJ, Voskuyl AE, Roos JC, Stigter vW, de Groot ER, Wolbink G 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(2):253-256.
- (9) Miyasaka N. Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study. *Mod Rheumatol* 2008; 18(3):252-262.
- (10) van der Bijl AE, Breedveld FC, Antoni CE, Kalden JR, Kary S, Burmester GR 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(8):1021-1028.
- (11) Radstake TR, Svenson M, Eijsbouts AM, van den Hoogen FH, Enevold C, van Riel PL et al. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 2008.
- (12) Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009; 137(5):1628-1640.
- (13) Sandborn WJ, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007; 56(9):1232-1239.

- (14) 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* 2008; 28(9):1122-1126.
- (15) Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; 130(2):323-333.
- (16) Lovell DJ, Ruperto N, Goodman S, Reiff A, Jung L, Jarosova K et al. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. *N Engl J Med* 2008; 359(8):810-820.
- (17) de Vries MK, Brouwer E, van der Horst-Bruinsma IE, Spoorenberg A, van Denderen JC, Jamnitski A et al. Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. *Ann Rheum Dis* 2009; 68(11):1787-1788.
- (18) 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(3):624-625.
- (19) Lecluse LL, Driessen RJ, Spuls PI, de Jong EM, Stapel SO, van Doorn MB et al. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. *Arch Dermatol* 2010; 146(2):127-132.
- (20) Wijbrandts CA, Dijkgraaf MG, Kraan MC, Vinkenoog M, Smeets TJ, Dinant H et al. 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* 2008; 67(8):1139-1144.
- (21) Marotte H, Arnaud B, Diasparra J, Zrioual S, Miossec P. Association between the level of circulating bioactive tumor necrosis factor alpha and the tumor necrosis factor alpha gene polymorphism at -308 in patients with rheumatoid arthritis treated with a tumor necrosis factor alpha inhibitor. *Arthritis Rheum* 2008; 58(5):1258-1263.
- (22) Bennett AN, Peterson P, Zain A, Grumley J, Panayi G, Kirkham B. Adalimumab in clinical practice. Outcome in 70 rheumatoid arthritis patients, including comparison of patients with and without previous anti-TNF exposure. *Rheumatology (Oxford)* 2005; 44(8):1026-1031.
- (23) Bombardieri S, Ruiz AA, Fardellone P, Geusens P, McKenna F, Unnebrink K et al. Effectiveness of adalimumab for rheumatoid arthritis in patients with a history of TNF-antagonist therapy in clinical practice. *Rheumatology (Oxford)* 2007; 46(7):1191-1199.
- (24) Solau-Gervais E, Laxenaire N, Cortet B, Dubucquoi S, Duquesnoy B, Flipo RM. Lack of efficacy of a third tumour necrosis factor alpha antagonist after failure of a soluble receptor and a monoclonal antibody. *Rheumatology (Oxford)* 2006; 45(9):1121-1124.
- (25) Astermark J, Oldenburg J, Pavlova A, Berntorp E, Lefvert AK. Polymorphisms in the IL10 but not in the IL1beta and IL4 genes are associated with inhibitor development in patients with hemophilia A. *Blood* 2006; 107(8):3167-3172.
- (26) Pavlova A, Delev D, Lacroix-Desmazes S, Schwaab R, Mende M, Fimmers R et al. Impact of polymorphisms of the major histocompatibility complex class II, interleukin-10, tumor necrosis factor-alpha and cytotoxic T-lymphocyte antigen-4 genes on inhibitor development in severe hemophilia A. *J Thromb Haemost* 2009; 7(12):2006-2015.
- (27) Huang DR, Zhou YH, Xia SQ, Liu L, Pirskanen R, Lefvert AK. Markers in the promoter region of interleukin-10 (IL-10) gene in myasthenia gravis: implications of diverse effects of IL-10 in the pathogenesis of the disease. *J Neuroimmunol* 1999; 94(1-2):82-87.

- (28) Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* 2007; 56(9):1226-1231.
- (29) Lichtenstein GR, Diamond RH, Wagner CL, Fasanmade AA, Olson AD, Marano CW et al. Clinical trial: benefits and risks of immunomodulators and maintenance infliximab for IBD-subgroup analyses across four randomized trials. *Aliment Pharmacol Ther* 2009; 30(3):210-226.
- (30) Burmester GR, Mariette X, Montecucco C, Monteagudo-Saez I, Malaise M, Tzioufas AG et al. Adalimumab alone and in combination with disease-modifying antirheumatic drugs for the treatment of rheumatoid arthritis in clinical practice: the Research in Active Rheumatoid Arthritis (ReAct) trial. *Ann Rheum Dis* 2007; 66(6):732-739.
- (31) Bennett CL, Luminari S, Nissenson AR, Tallman MS, Klinge SA, McWilliams N et al. Pure red-cell aplasia and epoetin therapy. *N Engl J Med* 2004; 351(14):1403-1408.
- (32) 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(12):3782-3789.
- (33) Vultaggio A, Matucci A, Nencini F, Pratesi S, Parronchi P, Rossi O et al. Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. *Allergy* 2010; 65(5):657-661.
- (34) de Vries MK, van der Horst-Bruinsma IE, Nurmohamed MT, Aarden LA, Stapel SO, Peters MJ et al. Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009; 68(4):531-535.
- (35) Jamnitski A, Nurmohamed MT, Hart MH, Dijkmans B, Aarden L, Wolbink G. Patients not responding to etanercept obtain lower trough etanercept concentrations compared to responding patients. *Arthritis Rheum* . 7-11-2010.
Ref Type: Abstract
- (36) Dore RK, Mathews S, Schechtman J, Surbeck W, Mandel D, Patel A et al. The immunogenicity, safety, and efficacy of etanercept liquid administered once weekly in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2007; 25(1):40-46.
- (37) Keystone EC, Schiff MH, Kremer JM, Kafka S, Lovy M, DeVries T 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(2):353-363.
- (38) Wolbink GJ, Aarden LA, Dijkmans BA. Dealing with immunogenicity of biologicals: assessment and clinical relevance. *Curr Opin Rheumatol* 2009; 21(3):211-215.
- (39) 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(1-2):82-88.

