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Immunogenicity of therapeutic antibodies

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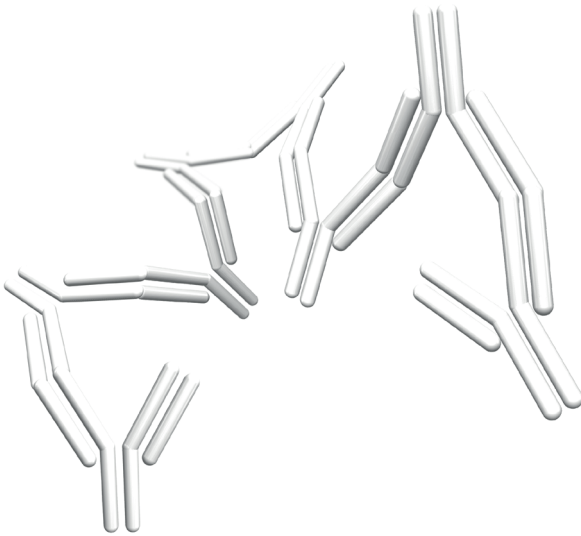
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Chapter 8

Summarizing discussion



Anti-drug antibodies

Immunogenicity is found to various degrees for many therapeutic antibodies, resulting in unwanted anti-drug antibody production by the patients. Decades of clinical experience has learned clinicians how to handle immunogenicity of antibody therapeutics. Through monitoring drug and ADA levels the efficacy of the drug is measured, and in case of non-response patients are switched to another therapeutic. Although this has led to effective treatment regimens, the underlying mechanisms, for instance how ADA reduce the drug level, are largely unknown.

The studies in this thesis focused on translating the unwanted clinical effects observed in patients with the immunological mechanisms behind these effects. These include the way in which ADA reduce the free drug level, how ADA may induce adverse events through complex formation, but also why complex formation in many ADA-positive patients goes by unnoticed. In this chapter, the combined results of all studies will be discussed.

The development of anti-idiotypic antibodies

The immune system has evolved to distinguish between self and non-self and is supposed to only respond in case of danger. Fully human therapeutic antibodies can be considered as 'self' and thus should not provoke an immune response. Nevertheless, many fully human therapeutics including monoclonal antibodies are found to be immunogenic.

Characterization of the antibody response towards therapeutic antibodies shows that ADA are generally class-switched and have undergone somatic hypermutation, indicating that this response is T-cell-mediated.¹⁻³ To obtain such a response, antigen presenting cells (APC) must have phagocytosed the drug and been activated by PRR-ligation in order to migrate to secondary lymphoid organs. There, APCs will have presented peptides to drug-specific CD4+ T-cells to induce differentiation of the latter. Additionally, drug specific B-cells must have been activated by BCR crosslinking. The exact sequence of events that initiate an anti-drug response is not fully known.

Activation of naïve B-cells can occur through antigen-driven crosslinking of BCRs. The exact requirements for BCR-mediated B-cell activation are not entirely clear. Some studies suggest that approximately 10-12 identical epitopes are necessary to provoke an antibody response,^{4,5} but others show that two or three epitopes⁶ or even monovalent antigens can induce B-cell activation.⁶ The latter two studies however, only analyzed BCR-induced intracellular signaling, and did not investigate antibody production. Furthermore, the threshold for B-cell activation also highly depends on other factors,

in particular costimulatory molecules including complement factors.⁷ Additionally, one could postulate that, due to the low affinity of the BCRs of naïve B-cells, multiple epitopes are required, simply because low valency antigens dissociate too quickly to provide proper activation.

As described in this thesis, therapeutic antibodies (including chimeric ones) are almost exclusively targeted on their idiotype, limiting the amount of BCR binding sites per antibody to two. Therapeutic antibodies are therefore presumably not able to sufficiently activate drug-specific naïve B-cells. Likewise, phagocytosis of monomeric antibody therapeutics by APCs is unlikely due to the lack of receptor binding and crosslinking, hence preventing activation of drug-specific T-cells. Therefore, at first sight, it seems that the ADA response observed in patients would not be induced by monomeric therapeutic antibodies.

However, multimers of therapeutic antibodies can be present in various ways. Aggregation of a therapeutic product is found to be a major contributing factor of immunogenicity in animal studies.^{8,9} Aggregation may take place during the many antibody production steps or during storage (e.g. due to shaking of vials, freezing or heating),¹⁰ and manufacturers take great care to prevent this. However, especially in patients that chronically use high dosages of therapeutic antibodies, elimination of any type of aggregation is challenging.

Apart from aggregation caused by production and storage, antibody complexes may also form *in vivo*. The most evident way is via target binding; for example binding of anti-TNF therapeutics to TNF. As discussed in **Chapter 3**, each trimeric TNF molecule can be bound by three anti-TNF antibodies, thus forming a trimeric complex. These trimers are likely phagocytosed, albeit with low efficiency, and peptides of the complex will be presented.

Next to soluble targets, monoclonal antibodies may also bind cellular targets. Although these might not be defined as immune complexes, they do facilitate phagocytosis. Cell depleting therapeutics will be cleared along with the opsonized target cell, and receptor blocking antibodies that do not induce cellular phagocytosis will eventually be cleared once the cell has reached its maximum life span. Phagocytosis by APCs and subsequent presentation to drug-specific T-cells is therefore likely a natural event for therapeutic antibodies. Thus, in case T-cell epitopes are present in the therapeutic product, T-cell activation seems not to be a limiting factor for immunogenicity, even without aggregation of the original product. Furthermore, the eventual production of

ADA may promote complex formation, phagocytosis and peptide presentation and could boost the initial immune response.

Activation of naïve B-cells by *in vivo* formed complexes may be a much more complicated event. Since the response towards antibody therapeutics is highly restricted to the idiotype, the BCR binding site is shielded once the drug binds its target, greatly limiting the amount of free epitopes for BCR crosslinking. For instance for the TNF:anti-TNF trimers, three Fabs are involved in target binding and three Fabs are exposed, and these complexes are presumably too small to activate naïve B-cells. However, once drug-specific B-cells are activated, for instance by aggregates in the product, and the BCR affinity has increased, TNF-anti-TNF trimers may induce crosslinking and thus boost the anti-drug response.

Therapeutic antibodies against cellular targets may activate B-cells via a quite distinct route compared to antibodies towards soluble targets. Complexes of antibodies and their soluble targets will probably come in contact with drug-specific B-cells through diffusion via the lymphatic system. With the exception of antibodies towards cells residing in the lymph node (e.g. natalizumab), antibody-coated cells are unlikely to take this route, thus preventing interaction with drug-specific B-cells.

A recent study by Heesters and colleagues¹¹ however, showed that complement-coated immune complexes could be transported to the lymph node by non-cognate B-cells via complement receptor 2, to be delivered to follicular dendritic cells. These cells were then found to internalize the complexes, after which they were recycled to the membrane while still bearing complement components, to be presented to cognate B-cells.

This mechanism might also be applicable for antibody-opsonized cells and could explain, at least in part, the B-cell activation found towards cell-targeting antibodies. Therapeutics inducing complement dependent cytotoxicity (CDC) coat the target cell with complement components. Similarly, targeted cells that go into (drug-mediated) apoptosis are presumably coated with complement as well.^{12,13} Either through lysis or via apoptotic blebbing, fragments of the complement- and drug-coated membrane of the target cell will be released. In a similar way as described by Heesters and colleagues, these fragments could be presented to drug-specific B-cells, assuming that not all Fabs of the drug are occupied. However, it remains to be determined whether antibodies stay attached to the complex during the recycling process, since in contrast to complement components, these are not covalently bound. Similar experiments as described by Heesters and colleagues could be performed with drug-coated target

cells to determine whether this scenario occurs *in vitro* and *in vivo*, possibly uncovering a piece of the anti-idiotypic puzzle.

In conclusion, some form of aggregation or complex formation is required to obtain the class-switched ADA response as observed in patients. Target binding may on the one hand facilitate phagocytosis and subsequent T-cell activation, but on the other hand impair B-cell activation by shielding the BCR binding site. Multiple mechanisms are possible that eventually induce B-cell activation and ADA production, but the exact events remain to be determined.

Neutralization and clearance

Antibody formation towards therapeutic antibodies is strongly associated with lower drug levels, but little is known about the mechanism that causes this association. Two frequently proposed mechanisms include neutralization and increased clearance due to complex formation. Previous work by our group (van Schouwenbrug et al.¹⁴) showed that the large majority of ADA to adalimumab competed with TNF for the antigen binding site, indicating that in this case, neutralization plays a significant role in lowering the drug concentration. Complementary to these results, **Chapter 4** and **Chapter 5** describe that the ADA response towards all anti-TNF therapeutics as well as to natalizumab is largely neutralizing. Thus, for all five investigated therapeutic antibodies the idiotype was found to be the predominant target of the ADA response, even in case of chimeric and humanized antibodies that contain (many) murine determinants outside of the idiotype. This strong bias suggests that the idiotype of antibodies plays an immunodominant role, and it may therefore be expected that the ADA response towards other antibody therapeutics is directed to the idiotype as well. This would imply that the main mechanism behind the lower detectable drug levels in ADA positive patients is neutralization of drug by ADA.

Neutralization however, inherently means that immune complexes are formed between ADA and drug, which directly relates the process of neutralization to the second proposed mechanism of drug-level reduction, namely increased clearance. Clearance of ADA-drug complexes is poorly investigated, probably since it is challenging to analyze this effect *in vivo*, let alone in ADA positive patients. In **Chapter 7**, *in vitro* experiments with human monocyte-derived macrophages show that complexes of drug and recombinant ADA can be cleared, although the size greatly influenced the efficiency of clearance. Tetrameric and larger complexes were rapidly phagocytosed, while dimeric complexes were cleared much less efficiently. The complex size between a certain drug and ADA may thus affect the significance of immune complex-mediated clearance on maintenance of drug levels. Drugs that are administered subcutaneously likely form

small immune complexes, due to the suboptimal ratio and concentration of drug and ADA. Indeed, in adalimumab-treated ADA-positive patients dimers are found weeks after the last administration of drug, implicating that these very small complexes are not efficiently cleared. It is therefore likely that, for subcutaneously administered drugs, ADA predominantly lowers the drug level through neutralization, whereas clearance only modestly affects the drug level.

For intravenously (iv) administered drugs, the contribution of neutralization and clearance might be different. With iv administration the total amount of drug directly reaches the systemic circulation and is immediately bound by ADA. Depending on the concentration and ratio of both antibodies, complexes larger than dimers could be formed, after which these are rapidly cleared. Two studies with humans and cynomolgus monkeys investigated clearance of iv administered radiolabeled drug in ADA positive and ADA negative subjects.^{15,16} In ADA positive subjects, immune complexes the size of dimers but also bigger complexes were formed. In addition, a higher amount of radioactivity was measured in the liver and the spleen, as compared to the ADA negative subjects. Notably, in the patient study this increased radioactivity was only observed after 2 hours but disappeared after 24 hours, indicating that the clearance of these complexes occurs fast. This may explain the *ex vivo*-observed lack of tetramers and larger complexes in serum of ADA positive patients, even though these could be formed *in vitro*, as is described in **Chapter 7**. In case of intravenously administered therapeutic antibodies, it is likely that neutralization and complex-mediated clearance both play a significant role in reducing the drug level (see Figure 1).

Underestimation of the ADA response

Alongside the development of antibody therapeutics, considerable improvements have also been made in the assays to measure ADA. Although this has greatly expanded our understanding of immunogenicity, we may still fairly underestimate this immune response.

Initially, assays such as the bridging ELISA could only detect free ADA and as a consequence, only patients with very low or undetectable drug levels were found positive for ADA. Later, drug tolerant assays enabled the detection of ADA in complex with drug, sometimes drastically increasing the percentage of ADA positive patients. For instance, adalimumab was first thought to be immunogenic in around 15% of the patients, but drug tolerant assays revealed that over 55% of the patients was positive for ADA.^{17,18} One important aspect however, is that none of the assays can measure ADA-drug complexes that are already cleared. During treatment, high levels of drug are administered and formation of low levels of ADA will promptly result in dimeric or trimeric

complexes. Once these complexes are cleared, or even when they are only bound to FcγRs, they will be absent from the sample used to measure ADA. The possibility thus exists that adalimumab is not immunogenic in 55%, but in a still higher percentage of patients, if not in all.

Interestingly, for infliximab a much higher concordance was found between the drug tolerant and intolerant assays, as was determined in three individual studies.¹⁹⁻²¹ This supports the idea that adalimumab primarily forms dimeric immune complexes which are not cleared, whereas infliximab forms larger complexes that are rapidly removed from the circulation. This would furthermore mean that the underestimation of immunogenicity is even higher in infliximab-treated patients.

Nevertheless, studies on immunogenicity of adalimumab and infliximab are likely biased in several ways, hampering direct comparison between these drugs. First, a bias may be found in the time points that the samples were taken, which is at trough level. This differs significantly between adalimumab (2 weeks after last dose) and infliximab (8 weeks after last dose), giving infliximab-treated patients much more time to clear immune complexes. Additionally, the drug concentration may be much lower at trough level in infliximab compared to adalimumab-treated patients, reducing the drug interference in drug intolerant assays. Other general differences between therapeutic antibodies may be found in drug dosing, way of target binding, ADA and drug affinity, route of administration, characteristics of patients and diseases, and other still unknown factors. Thus, comparison of the immunogenicity of different drugs is complicated in many ways.

The degree of clearance via ADA-drug complexes therefore probably differs for each drug. Nevertheless, clearance of immune complexes will almost certainly cause an underestimation of the ADA level and thus of the total immunogenicity of many drugs.

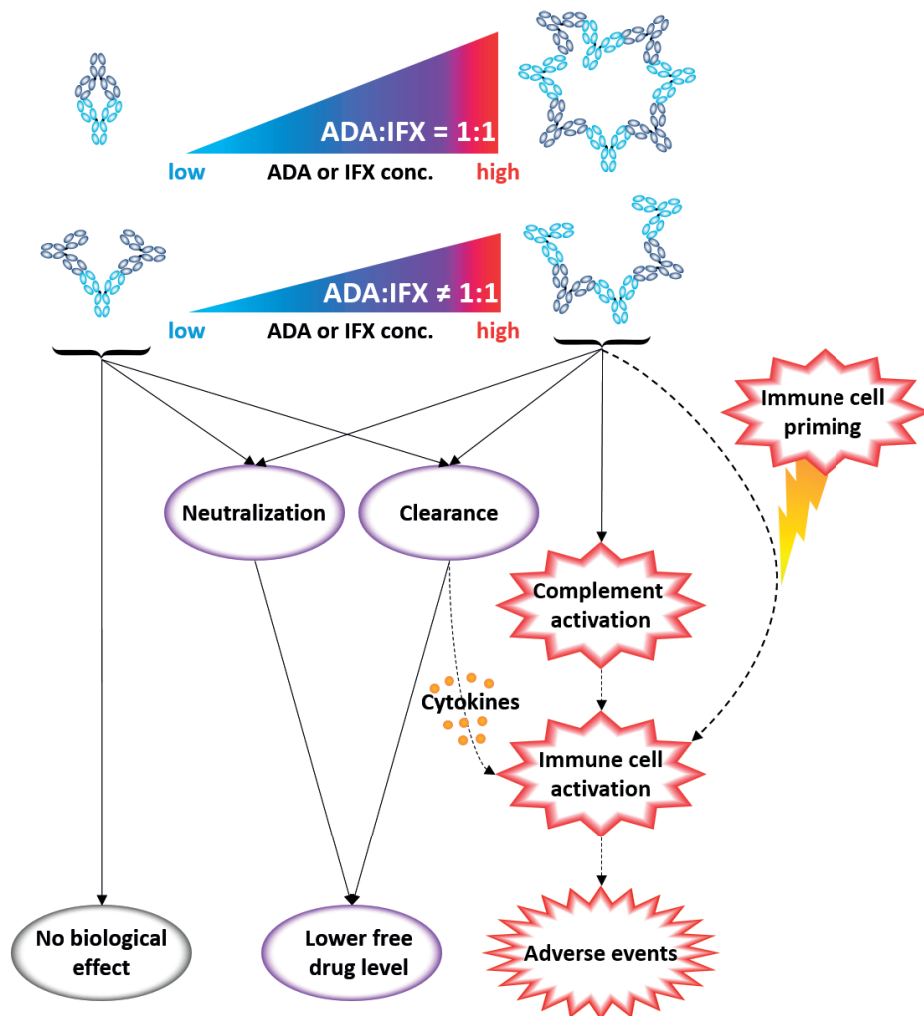


Figure 1. Schematic representation of the biological effects of anti-idiotypic immune complexes. Immune complex size is determined by concentration and ratio of both ADA and infliximab. Complex formation inherently neutralizes the drug, and furthermore facilitates clearance, thus lowering the free drug level. Small and moderate complexes have no biological effect, whereas very large complexes activate the complement system, and possibly further activate immune cells. Priming of immune cells by the pro-inflammatory status of the patient may possibly boost the activating potential of complexes. Furthermore, cells involved in clearance may produce pro-inflammatory cytokines, thus causing further activation. These various forms of immune activation may lead to an inflammatory state, causing ADA-induced adverse events.

Infusion reactions and other adverse events

Like all drugs, treatment with therapeutic antibodies may cause adverse events. Provoking an immune response against a therapeutic antibody is seen as such an unwanted effect. Moreover, ADA in itself may cause adverse events as well.

Infusion reactions are not mediated by IgE-ADA

One of the side effects that are associated with ADA positivity are infusion reactions, which are all adverse events that occur during intravenous administration of a therapeutic antibody. Infusion reactions are mainly observed during therapy with infliximab²²⁻²⁴ and natalizumab.^{25,26} The symptoms are very diverse, but largely resemble that of a hypersensitivity reaction. The role of IgE-ADA was therefore investigated by several groups,²⁷⁻²⁹ but the lack of a robust assay and a positive control complicated the interpretation of the results. In **Chapter 6**, a new assay is described to measure IgE-ADA. The common pitfalls of assay development, as described in **Chapter 2**, were solved and a positive control was constructed for assay validation. Using this assay, we found only a few IgE-ADA positive patients, and moreover their levels of IgE-ADA were generally very low. No association between infusion reactions and IgE-ADA to infliximab was found, implying that IgE-ADA is not the main cause for infusion reactions.

Currently, treatment protocols sometimes include pretreatment with antihistamines to prevent the occurrence of adverse events towards infliximab, assuming that these reactions are type I hypersensitivities. However, randomized controlled trials determining the efficacy of antihistamines as premedication are lacking.³⁰ In fact, many retrospective and prospective studies even show an increased chance of infusion related reactions after antihistamine pretreatment,³¹⁻³³ although beneficial effects have also been shown.³⁴ Since type I hypersensitivity can now be largely excluded as the cause for infliximab-induced infusion reactions, it asks for reexamination of the use of antihistamines to prevent or treat these reactions.

However, care must be taken with extrapolating these results to other therapeutic antibodies that cause hypersensitivity reactions, since isolated cases of IgE-mediated reactions have been described. A classic example of this are the anaphylactic reactions observed in several cetuximab-treated patients, caused by pre-existing IgE anti- α -gal antibodies that target this sugar moiety expressed on cetuximab.³⁵

Anti-idiotypic complexes may have differential clinical effects

The association between IgG-ADA and infusion reactions led us to the hypothesis that infusion reactions are mediated by large immune complexes between drug and ADA (see Figure 1). Large quantities of ADA in the blood circulation encountering

large quantities of drug during treatment could potentially lead to a massive amount of immune complexes, activating the immune system and thus causing side effects. As described in **Chapter 7**, indeed complexes the size of dimers, tetramers, hexamers, octamers and even larger complexes could be formed using monoclonal and polyclonal ADA. Only the very large complexes (likely bigger than octamers) however, were able to activate complement, and complexes were not able to induce IL-6 production in whole blood cultures. These results suggest that the vast majority of ADA-drug immune complexes will not activate the immune system and thus will not cause side effects. Only in rare situations, anti-idiotypic immune complexes may inflict unwanted clinical effects. Patients with very high ADA levels that are treated with intravenously administered drugs in an approximately equimolar ADA:drug ratio may form very large, irregularly shaped complexes that could activate the complement system. The biological activity of complexes may furthermore be influenced by their quantity and the subclass of ADA and drug.

Complexes of ADA and other therapeutic proteins

Immunogenicity is not only observed for therapeutic antibodies, but is found for many other therapeutic proteins, possibly also leading to immune complex formation. For instance, in about a third of the hemophilia A patients, replacement therapy with Factor VIII (FVIII) is found to be immunogenic.³⁶ The polyclonal anti-FVIII antibodies target multiple non-overlapping epitopes on FVIII simultaneously, indicating a broad response and thus resulting in immune complex formation.³⁷⁻³⁹ Nevertheless, immunogenicity is, apart from loss of response, not associated with an increase in adverse events. This might be explained by the low plasma concentration of FVIII (<1.5 nM,⁴⁰ corresponding to <0.5 µg/ml), which on one hand could inhibit the formation of large complexes, and on the other hand keeps the amount of complexes limited.

Other protein therapeutics that are frequently found to be immunogenic are interferon (IFN) alpha and IFN-β. Depending of the type of product, about 10-40% of the patients produce anti-IFN antibodies.⁴¹ A discrimination is sometimes made between binding antibodies (Bab) and neutralizing antibodies (Nab). BAb to IFNβ for instance, do not inhibit the working mechanism of the therapeutic, whereas Nab are found to reduce the clinical efficacy.⁴² These Bab however, precede the development of Nab,⁴² and the possibility therefore exists that the discrimination between the two largely rests in their affinity and quantity. A study on the binding sites of Nab and Bab using linear peptide stretches of IFNβ indeed found identical binding sites for both antibodies.⁴³ However, this study did not resolve the amount of antibody binding sites on the three-dimensional structure of IFNβ, and it thus remains unknown what type of complexes can be formed. Since the administered IFNβ concentration is generally low, and immunogenicity has not

found to increase the incidence of adverse events,⁴⁴ it is likely that only small complexes are formed.

The formation of high quantities of large immune complexes thus seems to be predominately occurring with monoclonal antibodies, and only in rare cases of high titers and intravenous administration. It is however likely that anti-idiotypic complexes alone are not sufficient to induce adverse clinical effects. High ADA levels do not inevitably cause adverse events - many patients with high ADA titers do not have any side effects - suggesting that other factors contribute.

Antibody isotype

In patients, prolonged exposure to therapeutic antibodies seems to skew the ADA response towards the IgG4 type, as was found for adalimumab and natalizumab.^{1,2} IgG4 antibodies are known to exchange half molecules, thus forming bispecific antibodies.⁴⁵ This could possibly reduce the immune complex size; exchanged ADA can only bind the drug with one Fab arm, thus blocking further attachment of drug to the complex. In addition, IgG4 antibodies are poor complement activators and have a lower affinity for FcγRs compared to IgG1, thereby reducing the immune activating capacity of complexes. Nevertheless, the IgG4 isotype of natalizumab does not appear to protect against adverse events.^{25,26} It should thus be further investigated to what extent IgG4-ADA has the potential to reduce the biological effects of immune complexes, and what their relationship is with the occurrence of infusion reactions.

Genetic factors may contribute to adverse events

A genetic association was found between infusion reactions and polymorphisms in the gene encoding for FcγRIIIb.⁴⁶ Infusion reaction positive patients had significantly more often NA1/NA1 alleles, compared to patients without these reactions. The same study furthermore showed that infusion reactions were associated with ADA formation, but unfortunately they did not calculate the covariance between positivity for the NA1/NA1 alleles and immunogenicity. The NA1 variant of FcγRIIIb has a higher affinity for IgG1 and IgG3 than the NA2 variant, leading to more efficient phagocytosis.⁴⁷ As FcγRIIIb is almost exclusively present on neutrophils, this could increase the pro-inflammatory effect of immune complexes on these cells. Recently, the same group showed that this polymorphism was also significantly more frequent in adalimumab-treated patients with systemic side effects, but the study included only a low number of patients.⁴⁸ Priority now lies on determining whether this polymorphism operates as an independent variable or that immunogenicity biases these results.

Immune cell priming could potentiate the immunological effects of complexes

Even within individual patients, factors contributing to adverse events may fluctuate in time. When looking retrospectively at the ADA levels of infusion reaction positive patients, about half of the patients had similar (high) ADA levels on earlier trough level measurements, whereas these infusions did not elicit adverse events (unpublished data).

The reason for this difference is unknown, but an explanation might be found in the state of disease activity. Since high ADA levels are strongly linked to reduced clinical response or even loss of response, these patients might be treated suboptimally. However, the diseases that are treated with natalizumab (MS) and infliximab (IBD, rheumatic diseases) are characterized by an unstable disease activity. Periods of low levels of inflammation or even remission alternate with flares of high disease activity. Loss of response to the antibody therapeutic may thus not become immediately visible. The expression of pro- and anti-inflammatory cytokines by immune cells, and possibly also activation of the complement system is closely linked to the fluctuations of the disease.⁴⁹⁻⁵² In RA and IBD, dysregulation of the immune system even precedes the clinical manifestations, and patients may be in a pro-inflammatory state without showing any symptoms.^{50,51}

Over time, an ADA positive patient may thus show significant variation in its inflammatory profile. Possibly, these mediators could prime the immune system into a pro-inflammatory and active state.⁵³ The additional high amount of large immune complexes that form upon treatment may then have differential effects in primed and unprimed patients, leading to a different clinical outcome regarding side effects.

Interestingly, the effect of soluble immune complexes on primed neutrophils have been investigated by several groups. It was found that immune complex deposition on the endothelium increased TNF-primed neutrophil adherence to the vascular wall, as compared to unprimed neutrophils.⁵⁴ Furthermore, GM-CSF-primed neutrophils were found to strongly respond to soluble immune complexes leading to release of lactoferrin, myeloperoxidase and reactive oxidant species (ROS), whereas unprimed neutrophils did not respond at all.⁵⁵ Apart from neutrophils, priming may also affect macrophages and fibroblasts,^{56,57} although the effect of immune complexes on these cells has not been investigated extensively.

Priming with inflammatory mediators may thus alter the response to immune complexes. It might prove worthwhile to investigate the effect of anti-idiotypic immune complexes on primed neutrophils or on neutrophils from patients with active inflammatory diseases. This could provide new insight into the *in vivo* differential effect of immune complexes.

Not all infusion reactions are ADA-mediated

It is important to notice that not all infusion reactions are inflicted by the drug itself. Placebo-controlled studies on natalizumab and infliximab show that infusion reactions are also observed in placebo-treated patients.⁵⁸⁻⁶⁰ The broad definition of infusion reactions may be the cause of this, since any adverse event that occurs during infusion (either placebo or drug) is classified as an infusion reaction. The symptoms described are very diverse and vary from mild (headache, fatigue, nausea) to severe (respiratory symptoms, serious allergic reactions).^{58,59} Apparently a considerable part of the predominantly mild and moderate adverse events observed during infliximab infusion are caused by other factors than the drug. This could be for instance the medical procedures themselves or psychological effects, although there are little or no studies done on these causes for infusion-related adverse effects.

Drug-induced infusion reactions can possibly be distinguished from those that are not drug-induced by the difference in type of symptoms. Considerably more symptoms of hypersensitivity (e.g. hypotension, urticaria) are observed in infliximab or natalizumab-treated patients compared to placebo-treated patients.^{58,60} Taking into account that ADA positive patients have a significantly higher chance of developing an infusion reaction,^{25,61} it is likely that the actual drug-induced adverse events are indeed Type III hypersensitivity reactions.

Immunogenicity of next generation antibodies

Research in this thesis has focused on the antibody response towards monospecific antibodies. As explained in the introduction, next generation antibody therapeutics may significantly deviate from this format. Immunogenicity of these next generation antibodies could therefore considerably influence the immune complex formation and alter their effector functions. Although the occurrence of these unwanted effects has not been extensively investigated, at least in part because these drugs are only recently being approved for clinical use, some predictions can be made.

Bispecifics

Bispecific antibodies are mainly developed to treat various types of cancer, and function through physically linking cancer cells to immune cells. The two distinct idiotypes per bispecific molecule inevitably also increases the amount of possible T-cell epitopes, and during development extra care should be taken to prevent this. In contrast, the two distinct idiotypes of these antibodies may protect against the formation of large immune complexes. ADA towards only one idiootype will result in small trimeric complexes, and larger complexes can only be formed in case of (equal) responses to both idiotypes.

Nevertheless, ADA towards only one idiotypes is sufficient to strongly impair the linking-mechanism of the drug.

Human(ized) bispecific antibodies are not yet approved for clinical use, and predictions on their immunogenicity are tricky due to their possibly altered way of B-cell and T-cell activation, and the improved general knowledge on reducing immunogenicity. However, the rat/mouse-bispecific antibody catumaxumab has been approved as cancer treatment. The majority of patients were found to develop ADA,^{62,63} but most interestingly, ADA positive patients responded significantly better to the treatment than ADA negative patients.⁶² The authors suggested that ADA-development reflected a stronger overall humoral immune response, which may be beneficial for the patients and might explain the results. Further analysis of the immune response however, suggests that (part of) the ADA are not directed at the idioype, but at framework regions of mouse IgG.⁶² A second explanation could therefore be that ADA bind to the cell-bound catumaxumab, thereby improving the opsonization of target cells.

The murine and rat origin of catumaxumab thus likely provokes a broad ADA response, possibly leading to large immune complexes. Nevertheless, a case study on readministration of catumaxumab showed that this was safe. Possibly, the intraperitoneal use of this bispecific might have prevented systemic adverse reactions.

Altered Fc

Altering the effector functions of the drug presumably also affect the effector functions of immune complexes in case of immunogenicity. Mutations of the Fc are described that remove all FcγR binding and complement activation.⁶⁴ ADA towards these drugs could result in immune complexes that have only half of the immune activating activity, thus protecting against ADA-mediated side effects. On the contrary, the clearance of these complexes may also be impaired, possibly leading to harmful immune complex deposition.

Improving effector functions, for instance by introducing a mutation to increase C1q binding⁶⁵ or by altering the glycosylation for stronger FcγR binding⁶⁶ could either promote or reduce their immunogenicity. On one hand, it may lead to increased phagocytosis and stronger T-cell help, but on the other hand it could reduce BCR crosslinking due to the faster clearance of immune complexes. In case ADA are formed, these therapeutics may potentiate the effects of the formed immune complexes, possibly causing more often or more severe adverse effects. Altering the Fc furthermore introduces novel determinants that are potentially immunogenic, possibly resulting in a broader antibody response. Results described in this thesis however, demonstrate an immunodominant

role for the idiotype, even in case of chimeric antibodies, making the occurrence of such a broad response less likely.

Antibody-drug conjugates

For cancer treatment, antibody-drug conjugates (ADC) are being developed in which toxic compounds are linked to tumor-specific antibodies, thereby concentrating the toxic effects on the tumor cells. Only few ADC are approved for clinical use, but their immunogenicity has been extensively investigated.⁶⁷ An example of this is the therapeutic ado-trastuzumab emtansine, which is found to be immunogenic in 5.3% of the patients.⁶⁸ Interestingly, the majority of the ADA response was found to target the linker between antibody and drug, as well as other neoepitopes. Results from the first clinical trials show no increase in adverse events in ADA positive patients.⁶⁸

The results from this study show that introducing neoepitopes (including those introduced by attaching the linker) may be equally or even more immunogenic than the idiotype. For ADC, immune complex formation might thus not only be determined by the anti-idiotype response, but also by the amount of neoepitopes, and to what extent these can be targeted regarding steric hindrance.

The presence of a toxic molecule furthermore complicates the prediction of immunogenicity and their associated effects. Next to tumor cells, also drug-specific B-cells are bound by the ADC, possibly eliminating these B-cells before they can become plasma cells. This however, clearly does not occur in all patients. Additionally, off-target toxic effects may occur on cells involved in clearance of the tumor cells, as well as on those that clear complexes between ADA and ADC. This could furthermore impair the presentation of tumor antigens, but also of ADC peptides.

In conclusion, some predictions on the occurrence of immunogenicity and their downstream effects can be made for next generation antibodies. However, their true effects will remain uncertain, and animal experiments and clinical studies will be required to elucidate the actual (unwanted) effects of these therapeutics.

Final recommendations

The combined results from this thesis provide a better understanding of the immunological mechanisms behind the clinical effects observed during immunogenicity of monoclonal antibody treatment. This leads to several recommendations for antibody developers and for the clinic, as will be described below.

The concentration of drug and ADA as well as the ratio in which these two are mixed were found to strongly influence the immune complex size, and possibly also influence their associated adverse events. Since high concentrations of both antibodies in a (near-)equimolar ratio resulted in the largest complexes, reduction of immune complex-mediated adverse effects may be achieved by either lowering the drug dose or by preventing the occurrence of an equimolar ratio. Although lowering the drug dose is not always desirable, the achieved ratios could alter by choosing a different route of administration, thereby influencing the formation and location of immune complexes. Local administration is thus preferred over intravenous administration to prevent systemic effects. This should be kept in mind during the development of new therapeutics, especially for those that are expected to be immunogenic. Nevertheless, most favorable is preventing (strong) immunogenicity in the first place.

Furthermore, investigation of the possible causes of adverse events upon infliximab treatment showed that the majority of these reactions are not IgE-ADA-mediated. IgG-ADA however, were already linked to these adverse events in infliximab (and natalizumab) treated patients, and results from this study suggest that this might be due to systemic complement activation. Nevertheless, these results require further investigation, preferably using samples of patients taken during or short after the occurrence of these adverse effects.

The results from these studies additionally suggest that the current treatment and pretreatment to reduce infliximab-induced adverse events could be optimized. As discussed above, antihistamine pretreatment often increases instead of decreases the incidence of adverse events. The lack of clinically relevant IgE-ADA in infliximab-treated infusion reaction patients contributes to the notion that antihistamines may not be effective.

If clinical data indeed confirm the role of complement activation in infusion reactions, possible alternatives for antihistamine treatment might target the complement system, for instance using C1-inhibitor. This treatment exclusively inhibits the classical pathway, which is desired since infliximab treatment already increases the susceptibility for infections. Additionally, the rapid phagocytosis of the largest and thus most immune activating complexes permits the use of a complement inhibitor with a short half-life, further minimizing the chance of infections. Various therapeutic variants of C1-inhibitor are currently in clinical use, including ones with a short half-life of 1.5 hours.⁶⁹ However, also C1-inhibitor can be immunogenic.

Nevertheless, the most favorable way of reducing ADA-mediated effects, including reduction of the free drug concentration and induction of adverse events, is to stop treatment with this specific drug and if possible, switch to another.

REFERENCES

1. Lundkvist M, Engdahl E, Holmen C, et al. Characterization of anti-natalizumab antibodies in multiple sclerosis patients. *Mult. Scler. J.* 2012;
2. van Schouwenburg PA, Krieckaert CL, Nurmohamed M, et al. IgG4 production against adalimumab during long term treatment of RA patients. *J. Clin. Immunol.* 2012;32(5):1000–6.
3. Kosmač M, Avčin T, Toplak N, et al. Exploring the binding sites of anti-infliximab antibodies in pediatric patients with rheumatic diseases treated with infliximab. *Pediatr. Res.* 2011;69(3): 243–248.
4. Sulzer B, Perelson AS. Immunons revisited: Binding of multivalent antigens to B cells. *Mol. Immunol.* 1997;34(1):63–74.
5. Dintzis HM, Dintzis RZ, Vogelstein B. Molecular determinants of immunogenicity: the immunon model of immune response. *Proc. Natl. Acad. Sci. U. S. A.* 1976;73(10):3671–5.
6. Volkman C, Brings N, Becker M, et al. Molecular requirements of the B-cell antigen receptor for sensing monovalent antigens. 2016;1–11.
7. Carroll MC, Isenman DE. Regulation of Humoral Immunity by Complement. *Immunity.* 2012; 37(2):199–207.
8. Hermeling S, Schellekens H, Maas C, et al. Antibody Response to Aggregated Human Interferon Alpha2b in Wild-type and Transgenic Immune Tolerant Mice Depends on Type and Level of Aggregation. *J. Pharm. Sci.* 2006;95(5):1084–1096.
9. Fathallah AM, Chiang M, Mishra A, et al. The Effect of Small Oligomeric Protein Aggregates on the Immunogenicity of Intravenous and Subcutaneous Administered Antibodies. *J. Pharm. Sci.* 2015;104(11):3691–3702.
10. Vázquez-Rey M, Lang DA. Aggregates in monoclonal antibody manufacturing processes. *Biotechnol. Bioeng.* 2011;108(7):1494–1508.
11. Heesters BA, Chatterjee P, Kim Y, Santiago F. NIH Public Access. 2014;38(6):1164–1175.
12. Mevorach BD, Mascarenhas JO, Gershov D, Elkon KB. Complement-dependent Clearance of. 1998;188(12.):
13. Hart SP, Smith JR, Dransfield I. Phagocytosis of opsonized apoptotic cells: Roles for “old-fashioned” receptors for antibody and complement. *Clin. Exp. Immunol.* 2004;135(2):181–185.
14. 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(1):104–9.
15. Rojas JR, Taylor RP, Cunningham MR, et al. Formation, Distribution, and Elimination of Infliximab and Anti- Infliximab Immune Complexes in Cynomolgus Monkeys. *J Pharmacol Exp Ther.* 2005; 313(2):578–585.
16. 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;66:253–256.
17. van Schouwenburg PA, Bartelds GM, Hart MH, et al. 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.

18. Bloem K, van Leeuwen A, Verbeek G, et al. Systematic comparison of drug-tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients. *J. Immunol. Methods.* 2015;418:29–38.
19. Vande Casteele N, Gils A, Singh S, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am. J. Gastroenterol.* 2013;108(6):962–71.
20. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut.* 2014;63(8):1258–64.
21. Kopylov U, Mazor Y, Yavzori M, et al. Clinical utility of antihuman lambda chain-based enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. *Inflamm. Bowel Dis.* 2012;18(9):1628–1633.
22. Park W, Yoo DH, Jaworski J, et al. Comparable long-term efficacy, as assessed by patient-reported outcomes, safety and pharmacokinetics, of CT-P13 and reference infliximab in patients with ankylosing spondylitis: 54-week results from the randomized, parallel-group PLANETAS study. *Arthritis Res. Ther.* 2016;18:25.
23. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm. Bowel Dis.* 2014;20(1):1–6.
24. Steenholdt C, Svenson M, Bendtzen K, et al. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 2011;34:51–58.
25. Calabresi PA, Giovannoni G, Confavreux C, et al. The incidence and significance of anti-natalizumab antibodies: Results from AFFIRM and SENTINEL. *Neurology.* 2007;69:1391–1403.
26. de la Hera B, Urcelay E, Brassat D, et al. Natalizumab-related anaphylactoid reactions in MS patients are associated with HLA class II alleles. *Neurol. Neuroimmunol. neuroinflammation.* 2014;1(4):e47.
27. Matucci A, Pratesi S, Petroni G, et al. Allergological in vitro and in vivo evaluation of patients with hypersensitivity reactions to infliximab. *Clin. Exp. Allergy.* 2013;43(6):659–64.
28. Vultaggio A, Matucci A, Nencini F, et al. Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. 2010;65:657–661.
29. Benucci M, Manfredi M, Saviola G, Baiardi P, Campi P. Correlation between atopy and hypersensitivity reactions during therapy with three different TNF-alpha blocking agents in rheumatoid arthritis. *Clin. Exp. Rheumatol.* 2009;27:333–6.
30. Lecluse LLA, Piskin G, Mekkes JR, Bos JD, De Rie MA. Review and expert opinion on prevention and treatment of infliximab-related infusion reactions. *Br. J. Dermatol.* 2008;159(3):527–536.
31. Choquette D, Faraawi R, Chow A, et al. Incidence and management of infusion reactions to infliximab in a prospective real-world community registry. *J. Rheumatol.* 2015;42(7):1105–1111.
32. Jacobstein D a, Markowitz JE, Kirschner BS, et al. Premedication and infusion reactions with infliximab: results from a pediatric inflammatory bowel disease consortium. *Inflamm. Bowel Dis.* 2005;11(5):442–446.
33. Wasserman MJ, Weber DA, Guthrie JA, et al. Infusion-related reactions to infliximab in patients with rheumatoid arthritis in a clinical practice setting: relationship to dose, antihistamine pretreatment, and infusion number. *J. Rheumatol.* 2004;31(6.):

34. Bartoli F, Bruni C, Cometi L, et al. Premedication prevents infusion reactions and improves retention rate during infliximab treatment. *Clin. Rheumatol.* 2016;35(11):2841–2845.
35. Arnold DF, Misbah S a. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N. Engl. J. Med.* 2008;358(25):2735; author reply 2735–6.
36. Peyvandi F, Ettingshausen CE, Goudemand J, et al. New findings on inhibitor development: from registries to clinical studies. *Haemophilia.* 2017;23:4–13.
37. Ananyeva NM, Lee TK, Jain N, Shima M, Saenko EL. Inhibitors in hemophilia A: Advances in elucidation of inhibitory mechanisms and in inhibitor management with bypassing agents. *Semin. Thromb. Hemost.* 2009;35(8):735–751.
38. Mühle C, Schulz-Drost S, Khrenov A V., et al. Epitope mapping of polyclonal clotting factor VIII-inhibitory antibodies using phage display. *Thromb. Haemost.* 2004;91(3):619–625.
39. Walter JD, Werther R a, Brison CM, et al. Structure of the factor VIII C2 domain in a ternary complex with two inhibitor antibodies reveals classical and non-classical epitopes. *Blood.* 2013; 122(26):4270–4278.
40. Butenas S, Parhami-Seren B, Undas A, Fass DN, Mann KG. The “normal” factor VIII concentration in plasma. *Thromb. Res.* 2010;126(2):119–123.
41. Strayer DR, Carter WA. Recombinant and natural human interferons: analysis of the incidence and clinical impact of neutralizing antibodies. *J. Interferon Cytokine Res.* 2012;32(3):95–102.
42. Creeke PI, Farrell R a. Clinical testing for neutralizing antibodies to interferon- β in multiple sclerosis. *Ther. Adv. Neurol. Disord.* 2013;6(1):3–17.
43. Gneiss C, Reindl M, Berger T, et al. Epitope specificity of neutralizing antibodies against IFN-beta. *J. Interferon Cytokine Res.* 2004;24(5):283–90.
44. Jones GJ, Itri LM. Safety and tolerance of recombinant interferon alfa-2a (Roferon-A) in cancer patients. *Cancer.* 1986;57(8 Suppl):1709–1715.
45. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin. Exp. allergy.* 2009;39:469–77.
46. Okuyama A, Nagasawa H, Suzuki K, et al. Fc γ receptor IIIb polymorphism and use of glucocorticoids at baseline are associated with infusion reactions to infliximab in patients with rheumatoid arthritis. 2011;299–304.
47. Salmon JE, Edberg JC, Kimberly RP. Fc γ receptor III on human neutrophils: Allelic variants have functionally distinct capacities. *J. Clin. Invest.* 1990;85(4):1287–1295.
48. Tsukamoto M, Kameda H, Ohshige T, et al. Fc γ receptor 3B polymorphism is associated with hypersensitivity reactions to adalimumab in Japanese patients with rheumatoid arthritis. *Mod. Rheumatol.* 2016;0(0):1–4.
49. Imam SA, Guyton MK, Haque A, et al. Increased calpain correlates with Th1 cytokine profile in PBMCs from MS patients. *J. Neuroimmunol.* 2007;190(1-2):139–145.
50. McInnes IB, Buckley CD, Isaacs JD. Cytokines in Rheumatoid Arthritis - Shaping the Immunological Landscape. *Nat. Rev. Rheumatol.* 2016;12(1):63–68.
51. Liverani E, Scaioli E, John Digby R, Bellanova M, Belluzzi A. How to predict clinical relapse in inflammatory bowel disease patients. *World J. Gastroenterol.* 2016;22(3):1017–1033.

52. Familian A, Voskuyl AE, van Mierlo GJ, et al. Infliximab treatment reduces complement activation in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 2005;64(7):1003–8.
53. Hallett MB, Lloyds D. Neutrophil priming: the cellular signals that say “amber” but not “green.” *Immunol. Today.* 1995;16(6):264–268.
54. Lauterbach M, O’Donnell P, Asano K, Mayadas TN. Role of TNF priming and adhesion molecules in neutrophil recruitment to intravascular immune complexes. *J. Leukoc. Biol.* 2008;83(6): 1423–1430.
55. Fossati G, Bucknall RC, Edwards SW. Insoluble and soluble immune complexes activate neutrophils by distinct activation mechanisms: changes in functional responses induced by priming with cytokines. *Ann. Rheum. Dis.* 2002;61(1):13–9.
56. Deng H, Maitra U, Morris M, Li L. Molecular mechanism responsible for the priming of macrophage activation. *J. Biol. Chem.* 2013;288(6):3897–3906.
57. Crowley T, O’Neil JD, Adams H, et al. Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. *Arthritis Res. Ther.* 2017;19(1):35.
58. Polman CH, O’Connor PW, Havrdova E, et al. A Randomized, Placebo-Controlled Trial of Natalizumab for Relapsing Multiple Sclerosis. *N. Engl. J. Med.* 2006;354(9):899–910.
59. Maini R, St Clair EW, Breedveld F, et al. Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet.* 1999;354(9194):1932–1939.
60. Schiff M, Keiserman M, Coddling C, et al. Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: a phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate. *Ann. Rheum. Dis.* 2008;67(8):1096–103.
61. 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(8): 1445–52.
62. Ott MG, Marm?? F, Moldenhauer G, et al. Humoral response to catumaxomab correlates with clinical outcome: Results of the pivotal phase II/III study in patients with malignant ascites. *Int. J. Cancer.* 2012;130(9):2195–2203.
63. Ruf P, Kluge M, Jäger M, et al. Pharmacokinetics, immunogenicity and bioactivity of the therapeutic antibody catumaxomab intraperitoneally administered to cancer patients. *Br. J. Clin. Pharmacol.* 2010;69(6):617–625.
64. Schlothauer T, Herter S, Koller CF, et al. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. *Protein Eng. Des. Sel.* 2016;29(10): 457–466.
65. Iduogie EE, Wong PY, Presta LG, et al. Engineered antibodies with increased activity to recruit complement. *J. Immunol.* 2001;166:2571–2575.
66. Yamane-Ohnuki N, Satoh M. Production of therapeutic antibodies with controlled fucosylation. *MAbs.* 2009;1(3):230–236.
67. Carrasco-Triguero M. Insights on the immunogenicity of antibody – drug conjugates. *Bionalysis.* 2015;7:1565–1568.

68. Carrasco-Triguero M, Yi J-H, Dere R, et al. Immunogenicity assays for antibody–drug conjugates: case study with ado-trastuzumab emtansine. *Bioanalysis*. 2013;5(9):1057–1071.
69. Sabharwal G, Craig T. Recombinant human C1 esterase inhibitor for the treatment of hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE). *Expert Rev. Clin. Immunol.* 2015; 11(3):319–327.