PREDICTABILITY OF ADVERSE REACTIONS TO BIOPHARMACEUTICALS

PhD thesis

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2 ABBREVIATIONS

ACE	Angiotensin converting enzyme
ADA	Anti-drug antibodies
ADR	Adverse drug reactions
αGal	Galactose-α-1,3-Galactose
BAT	Basophil activation test;
CD	Cluster of differentiation
CI	Confidence interval
CIOMS	Council of International Organization of Medical Sciences
CSF	Colony-stimulating factor
DNA	Deoxyribonucleic acid
DPT	Drug provocation test
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
EMA	European Medicines Agency
FDA	Food and Drug Administration
HER	Human epidermal growth
HLA	Human leukocyte antigen
HSR	Hypersensitivity reaction
GM	Granulocyte macrophage
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LAT	Lymphocyte activation test
LTT	Lymphocyte transformation test
NSAIDs	Non-steroidal anti-inflammatory drugs
mAb	Monoclonal antibody
MAH	Marketing authorization holder
MGDF	Megakaryocyte growth and development factor
MHC	Major histocompatibility complex
PBMC	Peripheral blood mononuclear cells

PEG	Polyethylene glycol
PD	Pharmacodynamic
PK	Pharmacokinetic
PML	Progressive multifocal leukoencephalopathy
Risk MAP	Risk minimization action plan
RMP	Risk management plan
ROC	Receiver-operated curves
RSV	Respiratory syncytial virus
SD	Standard deviation
SPT	Skin prick tests
TNF	Tumour Necrosis Factor
VEGF	Vascular endothelial growth factor
US	United States (of America)
WHO	World Health Organisation

3 INTRODUCTION (REVIEW OF LITERATURE)

3.1 Background on pharmacovigilance of biopharmaceuticals

3.1.1 Current trends in pharmacovigilance: focus on safety prediction

Pharmacovigilance is changing. It is no longer a passive discipline of awaiting and detecting adverse reactions, but active in predicting and managing risks. This approach is taken both by the European authorities in the form of risk management plans (RMP) and the United States Food and Drug Administration (US FDA) in Risk Minimization Action Plans (RiskMAPs). Even the very definition of pharmacovigilance is maybe no longer appropriate. It was not long ago in 2002 that the World Health Organization proposed the definition of pharmacovigilance as: "The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug related problem". Pharmacovigilance today must aim for more proactive approach to predict adverse effects and avoid them, and only detect and assess them where the predictive model fails or proves to be insufficient. While prediction is necessarily performed with a certain degree of uncertainty, a predictive model is indirectly recommended in RMPs and RiskMAPs. The level of uncertainty decreases with accumulating safety data throughout the product lifecycle and the predictive model is continuously refined. In lay terms, predictive model can be described as "educated guesswork" based on the thorough evaluation of available pre-clinical and clinical data on the medicinal product as well as the product class.

Industrial drug discovery aims at identifying drug candidates with the highest possible chance of completing clinical trials, reaching the market, and establishing themselves as efficacious and well-tolerated, safe medicines. Such drug candidates require a balance of favorable pharmacological, pharmacokinetic and physicochemical properties. Prediction of both efficacy (target effect) and safety (absence of off-target effect) starts in-silico. The absence of unintended pharmacological promiscuity, that is, the absence of interactions with non-therapeutic 'off-targets', is one important aspect of that balance. Pharmacological promiscuity can lead to adverse drug reactions (ADRs) and has been linked to preclinical findings of toxicity (2). ADRs and animal toxicity account for 30% of all drug candidate termination (3), and the proportion of promiscuous compounds decreases with advancing clinical development (4). The mainstay of pharmacological promiscuity assessment, however, is still the conventional screening against large panels of safety-relevant targets (2).

3.1.2 Immunogenicity and pharmacovigilance of biopharmaceuticals

Pharmacovigilance of biopharmaceuticals deals with all the complexities of conventional small molecule drugs, and on top of that, takes into account its own specificities. For biological drugs the task is, therefore, multiple-fold more complex.

Immunogenicity is the most typical adverse action of biopharmaceuticals. For some biopharmaceuticals this is not the most important adverse action (as demonstrated in the examples provided below), but essentially all biopharmaceuticals have been shown to exhibit some immune mediated adverse effect. Adverse reactions can be immediate – such as infusion reactions, or delayed – resulting from non-immediate action of anti-drug antibody (ADA) formation.

Infusion reactions are most commonly associated with a complex of chills, fever, nausea, asthenia, headache, skin rash, pruritus, etc. (5). However, infusion reactions may also present with a variety of signs and symptoms of severe hypersensitivity reaction. The mechanisms by which biopharmaceuticals elicit infusion reactions are multi-factorial. In addition to immune-mediated reactions, cytokine mediated effects are reported. For oncological therapy, tumour lysis syndrome should be considered in differential diagnosis of immune-mediated reactions. It is a syndrome in which the destruction of large numbers of rapidly proliferating tumour cells gives rise to hyperuricemia, hyperphosphatemia, and other metabolic abnormalities usually within 24 hours of infusion (5).

Monoclonal antibodies may interact with their molecular targets on circulating blood cells, tumour cells, or effector cells recruited to the tumour site (e.g., rituximab with cluster of differentiation (CD)20), thereby promoting the release of inflammatory cytokines. When released into the circulation, cytokines can produce a wide range of symptoms characteristic of infusion reactions (5). Because a cytokine-dependent mechanism does not depend on prior sensitization, it may contribute to infusion reactions that occur with the first infusion of a mAb. Massive cytokine release may precipitate life-threatening infusion reactions leading to multi organ failure, as in the case of a novel anti-CD28 monoclonal antibody (mAb). No evidence of anaphylaxis was seen (6).

Rituximab is characterised by an outstandingly high induction of infusion reactions compared to other biopharmaceuticals. During the first infusion to patients with relapsed B-cell chronic lymphocytic leukaemia or low-grade B-cell lymphoma, serum levels of Tumour

Necrosis Factor-α (TNF-α) and interleukin-6 (IL-6) peaked at 90 minutes and were accompanied by fever, chills, nausea, vomiting, hypotension, and dyspnoea. The severity of the infusion reaction was related to the number of circulating lymphocytes (7). It seems likely that the infusion reactions typical for rituximab are not due to its immunogenicity but due direct cytokine release.

Immediate-type (Type 1 or) hypersensitivity reactions are generally mediated by immunoglobulin E (IgE), leading to release of histamine, leukotrienes, and prostaglandins. These pro-inflammatory mediators induce smooth muscle contraction, capillary dilation, and vascular permeability, leading to the development of urticaria, rash, angioedema, bronchospasm, and hypotension. Anaphylaxis, the most severe form of immediate hypersensitivity, is a life-threatening condition that may appear within minutes of starting an infusion. It is characterized by respiratory distress, laryngeal edema, and severe bronchospasm, which may be accompanied by cutaneous and gastrointestinal symptoms, and may lead to a hypotensive crisis (8).

Because prior sensitization is required for immune-mediated hypersensitivity, it would not be expected to occur with the first administration. However, pre-existing IgE that cross-reacts with the drug may be responsible (9), as discussed below for cetuximab.

On the other hand, immunogenicity may not lead to immediate manifestations. Unwanted immunogenicity of erythropoietin leads to formation of neutralizing antibodies without demonstration of immediate type hypersensitivity (10).

It is well established that repeated injection of even native human proteins can result in a break in immune tolerance to self-antigens in some patients leading to a humoral response against the protein that is enhanced when the protein is aggregated or partially denatured (11). Although in some cases an immune response to a biopharmaceutical has limited clinical impact, ADAs may pose a number of potential risks for the patient. Firstly, an ADA response can adversely affect the pharmacokinetics and bioavailability of a drug thereby reducing the efficacy of treatment. But more importantly, ADAs can also adversely affect the safety of treatment and cause immune complex disease, allergic reactions and, in some cases, severe autoimmune reactions. Serious and life-threatening adverse events can occur when ADAs cross react with an essential non-redundant endogenous protein such as erythropoietin or thrombopoietin. Thus, several cases of pure red cell aplasia were associated with the development of antibodies to recombinant erythropoietin following a change in formulation

(10). Similarly, the development of antibodies to pegylated megakaryocyte growth and development factor (MGDF) cross reacted with endogenous MGDF resulting in several cases of severe thrombocytopenia (12).

In silico models have been used with several notable published successes in predicting immunogenicity of pharmaceuticals. In silico methods are based on the ability of T-helper cell recognition of antigenic epitopes. T-helper cells, a subset of T-lymphocytes specifically recognize epitopes presented by antigen presenting cells in the context of major histocompatibility complex (MHC) Class II molecules. T-helper cells, are the major drivers of the mature antibody response. Protein therapeutics that express MHC Class II restricted T-helper epitopes are likely to elicit more frequent and mature antibody responses with IgG as predominant isotypes. These T-helper epitopes can be represented as linear sequences comprising 8 to 12 contiguous amino acids that fit into the MHC Class II binding groove. A number of computer algorithms have been developed and used for detecting Class II epitopes within protein molecules of various origins. Such "in silico" predictions of T-helper epitopes have already been successfully applied in attempts to increase immunogenicity and efficacy of vaccines (13).

The relationship between T-cell epitopes and immune response has also been the subject of a number of investigations in the field of protein therapeutics. In some cases, therapeutic proteins have also been screened for T-helper epitopes in an attempt to evaluate their potential immunogenicity. Obviously, reliable in silico prediction of helper epitopes would be of significant value in development of protein therapeutics. Such predictions would make it possible to meaningfully rank candidates at the pre-clinical stage of drug development or to reengineer proteins to make them less immunogenic. Furthermore, individuals at higher risk of developing T-cell-driven antibody responses to the protein therapeutic could be identified prospectively using human leukocyte antigen (HLA) typing, if certain HLA can be associated with T-cell response and higher neutralizing antibody titres, as recently described by Barbosa et al (14).

Some in silico algorithms are freely available for public use on the internet. (http://www.pharmfac.net/allertop/, http://www.pharmfac.net/EpiTOP/). The validity of in-silico and other prediction methods still needs to be demonstrated on a wider scale even for small molecular entities. The use of these methods in the context of clinical trials of protein therapeutics is rather recent and deserves further exploration.

Animal data are considered not to be predictive for immunogenicity assessment of biopharmaceuticals, but they may be useful to detect major differences in immune response. For example, animal models may in some cases be of value for the comparative immunogenicity assessment of new product candidates. Such an example is chemically modified human factor VIII products for the treatment of haemophilia A developed with the aim of extending the half-life of Factor VIII. Because any chemical or molecular modification of a protein might create new immunogenic epitopes or generate structures that could stimulate the innate immune system, it is reasonable to compare their potential immunogenicity to the non-modified factor VIII molecule before entering clinical development. New mouse models of haemophilia were specifically designed for comparative immunogenicity assessment during preclinical development of modified factor VIII proteins. One of these models expresses a human factor VIII complementary deoxyribonucleic acid (c-DNA) as a transgene which causes the development of immunological tolerance to native human factor VIII. When immune-tolerant mice are treated with a modified human factor VIII that expresses new immunogenic epitopes, tolerance breaks down and antibodies against human factor VIII develop. Therefore, this model allows for the exclusion of high-risk candidates early during pre-clinical development. The other mouse model of haemophilia expresses a human MHC-class II protein that is associated with an increased risk for the development of antibodies against factor VIII in patients. As all murine MHC-class II genes are completely knocked out in this model, factor VIII peptides that drive anti-factor VIII immune responses are presented by the human MHC-class II protein. Although such models have their limitations, e.g. the human MHC-class II complex is usually highly polymorphic not consisting of only one or two haplotypes, they might help to identify high-risk candidates before entering clinical development (15). The final immunogenicity assessment, as in any predictive model, still requires clinical studies.

3.1.3 Factors influencing the immunogenicity of biopharmaceuticals

Currently available techniques do not permit one to predict with a sufficient degree of accuracy whether a biopharmaceutical will be immunogenic and if so, to what extent (16). It is also difficult to predict which patients will develop an immune response to a particular drug, and at what time during treatment an immune response will occur.

There are, however, a number of both drug-related and patient-related factors that are known

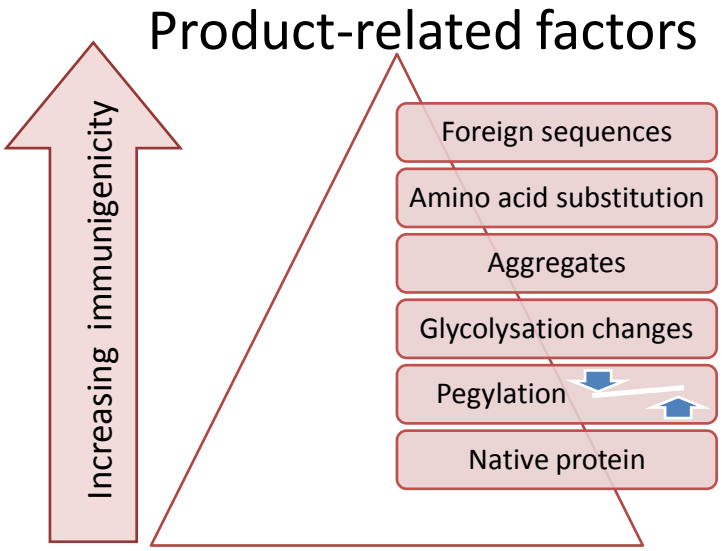
to influence the immunogenicity of biopharmaceuticals, as presented in Figure 1, . Drug-related factors include the presence of nonhuman sequences or novel epitopes generated by amino acid substitution designed to enhance stability, or novel epitopes created at the junction of fusion proteins. Molecular structure, and in particular, changes in glycosylation, can also influence the immunogenicity of a biopharmaceutical. Thus, the absence of glycosylation or an altered pattern of glycosylation can expose cryptic B-cell and T-cell epitopes in the protein, or cause the protein to appear foreign to the immune system (17).

Carbohydrate moieties present upon biopharmaceuticals can elicit the production of IgE antibodies that can cause serious adverse reactions including anaphylaxis even upon the first treatment exposure. Pre-existing antibodies against galactose-a-1,3-galactose (αGal) have been shown to be responsible for IgE-mediated anaphylactic reactions in patients treated with cetuximab (9). Pegylation can reduce the immunogenicity of some proteins although patients produce antibodies to the polyethylene glycol (PEG) residue adversely affecting efficacy (18).

In addition to attributes that can induce a classical immune response, repeated administration of even authentic human proteins such as albumin can under certain circumstances cause a break in immune tolerance leading to the development of an immune response. Thus, the presence of degradation products resulting from oxidation or deamination of the protein, aggregates, or the intrinsic immunomodulatory properties of the molecule can also influence the immunogenicity of a biopharmaceutical. Protein aggregation in particular has long been associated with increased immunogenicity, although the mechanisms underlying this effect remain poorly understood. It has been suggested that aggregated proteins form repetitive arrays that can lead to efficient cross-linking of B-cell receptors, leading to B-cell activation in the absence of T-cell help, thereby resulting in a break in immune tolerance to self-proteins (19).

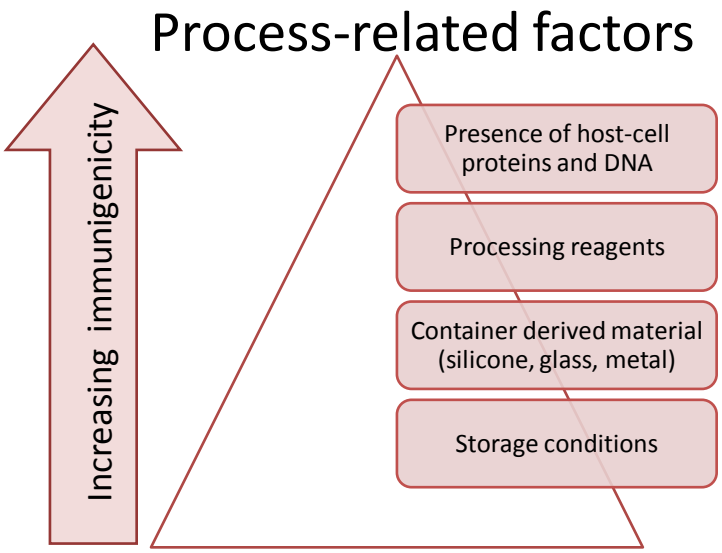
The relatively high incidence of ADAs in patients treated with recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) may be related at least in part to the immunostimulatory properties of the molecule itself (18). Thus, GM-CSF can recruit antigen-presenting cells to the site of antigen processing, stimulate the maturation of myeloid dendritic cells, and enhance an antigen-specific CD8+ T-cell response, suggesting that repeated administration of GM-CSF may function as an adjuvant. Indeed, GM-CSF has been used as an immunological adjuvant in a number of vaccination protocols designed to elicit an immune response to self-antigens (20).

Figure 1: Product-related factors affecting the immunogenicity of biopharmaceuticals



Process-related impurities, including traces of residual DNA or proteins from the expression system, or contaminants that leach from the product container, can also influence the immunogenicity of recombinant biopharmaceuticals (22).

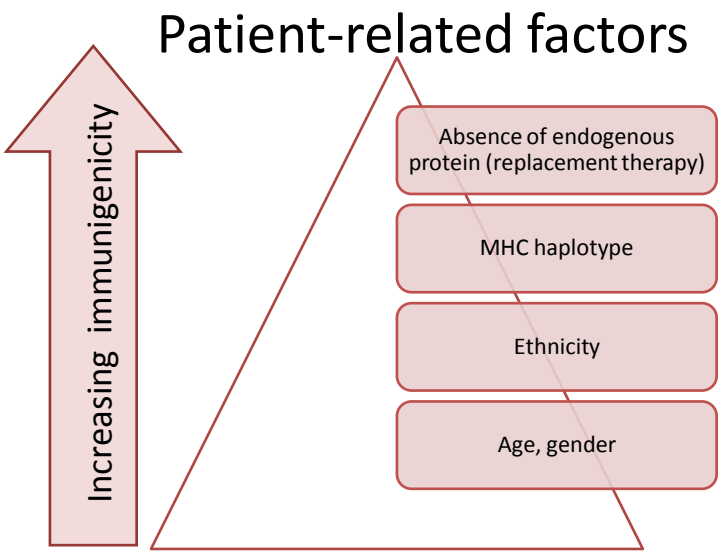
Figure 2: Process-related actors affecting the immunogenicity of biopharmaceuticals



Patient-related factors, such as genetic makeup, age, gender, disease status, concomitant medication, and route of administration, can also influence the immune response to a particular biopharmaceutical. For example, a common MHC class II allele, DRB1*0701, is associated with the antibody response to interferon-β in multiple sclerosis patients (17).

Disease state and immune competency also influence an individual's immune response to a treatment with a biopharmaceutical. Thus, development of antibodies to pegylated MGDF is less frequent in cancer patients who tend to be immunosuppressed than in healthy individuals (12).

Figure 3: Patient-related factors affecting the immunogenicity of biopharmaceuticals

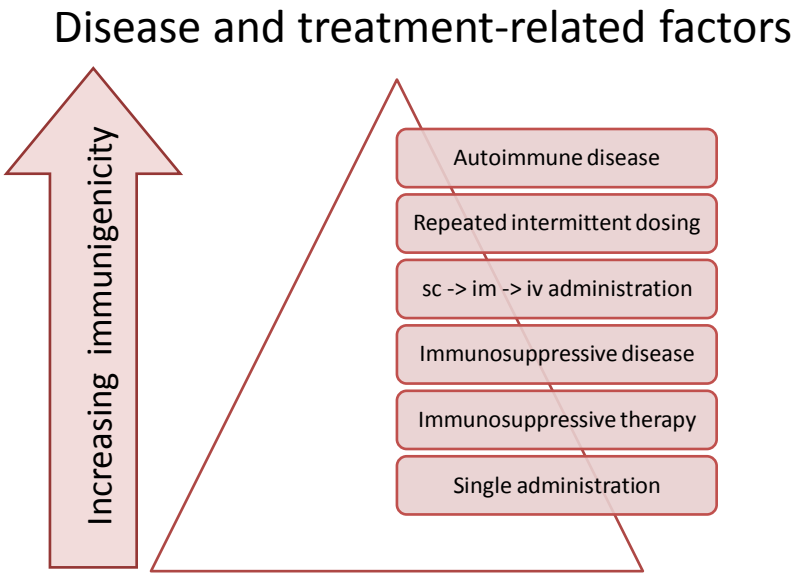


Concomitant therapy with immunosuppressive drugs can also influence a patient's immune response to a biopharmaceutical. Thus, administration of methotrexate together with the chimeric monoclonal antibody infliximab has been shown to reduce the immune response to infliximab and improve the clinical response in patients with rheumatoid arthritis (23). The duration of treatment and the route of administration also influence the immune response to a biopharmaceutical.

Typically, administration of a protein in a single dose results in the production of low-affinity IgM antibodies, while repeated administration results in the production of high-affinity and high-titer IgG antibodies, which may be neutralizing. Thus, in patients with multiple sclerosis treated with interferon-β neutralizing antibodies to IFNβ often do not appear until after several months of therapy (24).

The intravenous route of administration is considered to be least likely to generate an immune response to a biopharmaceutical compared with intramuscular or subcutaneous administration (15).

Figure 4: Disease and treatment-related factors affecting the immunogenicity of biopharmaceuticals



The complexity of the humoral response to biopharmaceuticals and the difficulty in establishing the effect on ADAs on drug efficacy is illustrated by the response of patients to treatment with IFNβ, for the treatment of relapsing remitting multiple sclerosis. Five products are currently available in the US and Europe as first-line disease-modifying agents for the treatment of relapsing remitting multiple sclerosis , IFNβ-1a (Avonex ® and Rebif®), IFNβ-1b (Betaseron® and Betaferon®), and more recently, the IFNβ-1b biosimilar Extavia®. Avonex® and Rebif® are both glycosylated forms of native human IFNβ-1a produced in Chinese hamster ovary cells. Betaseron® and Extavia® are a nonglycosylated form of IFNβ-1a produced in Escherichia coli that has a serine substitution for the unpaired cystine at position 17 of the native protein. Most patients develop an antibody response to IFNβ products, and as many as up to 45% of patients develop neutralizing antibodies to IFNβ, in some cases as early as 3 months after initiation of therapy. Overall, some 25% of patients develop anti-IFNβ-neutralizing antibodies usually within 6 to 18 months. ADAs are more frequent in patients treated with IFNβ-1b than IFNβ-1a, while subcutaneous IFNβ-1a (Rebif®) is more immunogenic than intramuscular IFNβ-1a (Avonex®) (32). The immunogenicity of IFNβ varies among individuals, both as a function of the presence of particular MHC class II alleles, and as a function of IFN-receptor expression. Thus, patients who process the DRB1*0701 allele, or who express low levels of IFNAR2, one of the two

chains of the type I IFN receptor, upon initiation of treatment, have a significantly higher risk of developing anti-IFNβ neutralizing antibodies (33). Although it has been shown in numerous trials that patients who develop antibodies against IFNβ have higher relapse rates, increased number of lesions detected by MRI, and higher rates of disease progression, the significance of anti-IFNβ ADAs remains controversial (34). This is due to the difficulty in establishing a temporal correlation between the presence of anti IFNβ ADAs and the loss of drug efficacy due to the variable nature of the disease, the partial effectiveness of the drug, the delay between initiation of treatment and the detection of an effect of the drug on the course of the disease, and the difference in the immunogenicity of different IFNβ products. The lack of standardized ADA assays has also rendered direct comparisons of immunogenicity between different products and different studies difficult, which has contributed to the difficulty in establishing a correlation between ADAs and loss of drug efficacy.

The assessment of efficacy described above for multiple sclerosis is complex enough, but still comparatively well-grounded in quantifiable and comparable assessment of relapse rate and the number of inflammatory lesions. On the other hand, the assessment of safety takes into account all of the complexities described above for efficacy, and additionally needs to account the relatedness, relevance and severity of reactions in the background rate of adverse events in the given population. Singling out adverse reactions which may be due to development of ADAs and other immunological mechanisms from the reactions due to target effect of the drugs seems like an unachievable aim.

3.1.4 Regulatory guidance

Assessment of immunogenicity is an important component of drug safety evaluation in preclinical, clinical, and post-marketing studies. Draft Guidance for Industry Assay Development for Immunogenicity Testing of Therapeutic Proteins has been published by the US Food and Drug Administration (35). Similarly, guidelines on the immunogenicity assessment of biotechnology- derived therapeutic proteins established by the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA) came into effect in April 2008 (36). These guidelines provide a general framework for a systematic and comprehensive evaluation of immunogenicity that should be modified as appropriate, on a case-by-case basis. Although differences in approach and emphasis exist between the US, European Union, and Japanese regulatory authorities there is, nevertheless, a large degree of

consensus on the type of approach that should be adopted; namely, a risk-based approach that is clinically driven and takes into account pharmacokinetic data. Thus, biopharmaceuticals with no endogenous counterpart are considered to be of relative low risk, while drugs with a non-redundant endogenous counterpart are considered to present a high risk. A multi-tiered approach to testing samples is also recommended. This consists of an appropriate screening assay capable of detecting both IgM and IgG ADAs, the sensitivity of which is such that a percentage of false-- positive samples would be detected. The specificity of the samples that test positive in the screening assay are then re-assayed in a confirmatory assay usually by competition with an unlabelled drug using the same assay format as that used for the screening assay. Samples that test positive in the screening and confirmatory assays are then tested for the presence of neutralizing ADAs using a cell-based assay whenever possible.

The prediction of both incidence and clinical significance of immunogenicity is still problematic. Therefore, the recommended approach is to apply suitably sensitive bioanalytical methods to detect host responses to the drug product and to relate these to clinical correlates of pharmacokinetics (PK), efficacy and safety. The current, commonly used, and in most cases recommended bioanalytical approach, is a three-stage process consisting of screening, confirmation, and characterisation: If blood samples are found to be positive for ADA during screening, these samples are then subjected to a confirmatory assay (e.g. competitive inhibition enzyme-linked immunosorbent assay (ELISA)) ensuring that ADA are binding specifically. Having established that positive findings do not result from non-specific interactions such as with materials in the assay milieu (e.g. plastic, other proteins), ADA need to be characterised. Typically, this characterisation includes assessment of ADA neutralising capacity. Furthermore, assays for relevant biomarkers and/or pharmacokinetic measurements should complement ADA characterisation and analysis of their in vivo impact (17).

Clinical consequences of immunogenicity may be comprised of acute consequences, such as anaphylaxis or infusion reactions, non-acute consequences (e.g. loss of efficacy), cross-reactivity with and neutralisation of natural endogenous counterparts, and delayed hypersensitivity. Therefore, the clinical outcome of unwanted immune responses differ widely depending on the affinity, class, amount and persistence of ADA generated, the epitope recognised by the biotherapeutic protein, and the ability of ADA to activate complement. This diversity of causes and consequences underlines the importance of the systematic evaluation of immunogenicity during clinical trials (15).

3.1.5 Biosimilars or similar biotherapeutic products

Biosimilar products present a specific challenge not only for immunogenicity assessment, but for safety and efficacy assessment overall. The potential for altered immunogenicity needs to be considered even if comparative physicochemical and biological data on product quality do not indicate any difference. Because the predictability of non-clinical studies for the evaluation of immunogenicity is low, routine monitoring of patient samples might be required during clinical trials. In this respect, the extent of immunogenicity studies (clinical evaluation prior to or after authorisation of the change) might be based on a risk analysis that pays regard to both the nature of any observed differences and their potential clinical impact. Emerging technologies might provide additional data for the further evaluation of potential immunogenicity induced by the change introduced in the process.

The current understanding is that the biosimilar and the innovator product should be identical on the aminoacid level and any difference needs to be justified, e.g. in posttranslational modifications. However, this approach is being challenged and current recommendations call for accepting highly similar to the reference medicinal product in physicochemical and biological terms. (36) This is formulated in draft guidelines released for public consultation by the EMA in 2013, but is subject to possible modification. For assessment of immunogenicity, human data are always required. Animal data, even if potentially useful to detect major differences in immune response, are considered not to be predictive. An optimised antibody testing strategy with detailed sampling protocols, a sensitive validated screening assay and further characterisation of ADA, if detected, is requested. (15)

Immunogenicity data (on both the biosimilar and the reference product) are usually required for interpretation of the results. The pre-licensing immunogenicity database is expected to exclude excessive immunogenicity of the biosimilar relative to the reference product (39) but further data may be requested post-marketing. According to the European Union guidelines on the immunogenicity assessment of biotechnology- derived therapeutic proteins: further systematic immunogenicity testing might become necessary after marketing authorization, and may be included in the RMP. This may be applicable in particular in situations when rare and serious ADA-related adverse reactions have been encountered with the reference product or the substance class. Because of these regulations, biosimilars in the European Union show close resemblance to their reference product with respect to quality, efficacy and safety. In this respect, global consistency is needed because ‘copy versions’ of innovator biologicals are

licensed in various other countries without a clear regulatory pathway and based on different data requirements.

It is important to use appropriate terminology for biosimilars. “Biosimilar” should be used in the context of a product meeting the regulatory requirements for a biosimilar within the given legislation. It should not be used for nay product which is similar or bears resemblance, but this has not been approved by a regulatory authority. Whereas the term biosimilar is used both by European legislator and the US FDA, it has to be emphasized that the term may be used only if such a product has gained approval. Therefore biosimilar according to European Union may not necessarily be biosimilar according to US standards, despite the harmonisation efforts which are being undertaken. In addition, various other legislations and organisations apply different terminologies. In Canada, for example, the term used is “subsequent entry biological” (40) whereas World Health Organisation (WHO) uses the term “similar biotherapeutic products” (41).

Due to the general lack of standardised assays, comparative immunogenicity data (on both the biosimilar and the reference product) are usually required for interpretation of the results. The pre-licensing immunogenicity database is expected to exclude excessive immunogenicity of the biosimilar relative to the reference product but further data may be requested post-marketing, especially when rare and serious ADA-related adverse reactions have been encountered with the reference product or the substance class. Because of these regulations, biosimilars in the European Union show close resemblance to their reference product with respect to quality, efficacy and safety. In this respect, global consistency is needed because ‘copy versions’ of innovator biologicals are licensed in various other countries without a clear regulatory pathway and based on different data requirements. Moreover, such non-innovator products are often called “biosimilars” despite the lack of a (thorough) comparison with the original product and even in the presence of clear differences. Therefore, WHO has developed the ‘Guidelines on evaluation of similar biotherapeutic products’ which in principle is in line with the European Union requirements (41).

3.2 Tests used for prediction of immunogenicity

In vivo and in vitro tests are used in diagnosis of drug hypersensitivity. In vivo skin tests such as prick, patch, and intra-dermal tests are the most readily available tools. Most readily refers to the ease of performing with the minimal need for use of specialized equipment and

reagents. Determination of specific IgE levels is still the most common *in vitro* method for diagnosing immediate reactions. New diagnostic tools, such as the basophil activation test, the lymphocyte activation test, and enzyme-linked immunospot assays for analysis of the frequency of antigen-specific, cytokine-producing cells, have been developed for evaluating either immediate or non-immediate reactions (42).

However, neither specificity nor sensitivity of allergologic tests is100%. Therefore in selected cases provocation tests (i.e. rechallenge with primarily diagnostic purpose) are necessary. As provocation testing caries a significant risk, it should be performed only when necessary.

In selecting diagnostic tests it is important to consider whether the reaction is immediate or non-immediate. The tests which were identified as currently most frequently used are summarized in Table 1 (42).

Table 1: Diagnostic tests of hypersensitivity reactions to drugs

Type of reaction	Type of tests	
Immediate	*In vitro*	Specific IgE assays
		Flow cytometric Basophil activation tests
	In vivo	Skin tests
		Provocation tests
Nonimmediate	*In vitro*	Lymphocyte transformation tests or Lymphocyte activation tests
		Enzyme-linked immunospot assays for analysis of antigen-specific, cytokine-producing cells
	In vivo	Delayed-reading intradermal tests
		Patch tests
		Provocation tests

Immediate reactions occur within the first hour after the last drug administration and are manifested clinically by urticaria, angioedema, rhinitis, bronchospasm, and anaphylactic shock. Non-immediate reactions occur more than 1 hour after the last drug administration. The main non-immediate reactions are maculo-papular eruptions and delayed-appearing urticarial exanthema. Immediate allergic reactions are thought to be IgE-mediated and have been extensively studied, whereas the mechanisms involved in non-immediate reactions seem

to be heterogeneous. However, clinical and laboratory studies indicate that a T cell–mediated pathogenic mechanism is often involved in macula-papular rashes. This mechanism has also been demonstrated in other non-immediate reactions, such as urticarial manifestations, angioedematous manifestations, or both; toxic epidermal necrolysis; bullous exanthems; drug reaction with eosinophilia and systemic symptoms; and acute generalized exanthematous pustulosis (43).

In non-allergic hypersensitivity reactions to drugs, inflammatory mediators are released by nonspecific immunologic mechanisms. The drugs most frequently responsible for such reactions are traditional small molecule drugs (e.g. non-steroidal anti-inflammatory drugs), with biological agents increasingly involved (44).

3.2.1 Skin tests

There is only a small number of drugs for which skin testing can provide useful information e.g. penicillin, muscle relaxants and carboplatin skin testing. However, for most drugs the relevant immunogen (intermediate metabolite) is unknown and therefore the predictive value of skin testing remains undetermined. Both false-positive and false-negative results may occur (45).

Skin prick tests (SPTs) for specific IgE-mediated drug reactions are useful for the diagnosis of reactions with both low molecular weight and high molecular weight agents. Tests are normally carried out at therapeutic concentrations unless the drug possesses intrinsic histamine-releasing activity in which case a dilution may be appropriate to avoid false-positive results (45).

Skin tests have to be applied according to the suspected pathomechanism of the drug hypersensitivity. An IgE-mediated reaction can be demonstrated by a positive skin prick and/or intradermal test after 20 min. On the other hand, non-immediate reactions to β-lactams manifesting by cutaneous symptoms occurring more than one hour after last drug intake, are often T-cell mediated and a positive patch test and/or a late-reading intradermal test is found after several hours or days. Moreover, skin tests have the additional capability to give insights concerning the immunologic pathomechanism (46).

There are other diseases where immunological reactions to drugs could be involved, but skin testing has generally not been found helpful. For example, renal or hepatic manifestations may occur as a part of a generalized allergic reaction (e.g., in “drug reaction with eosinophilia and systematic symptoms”). This is an obvious demonstration of the inappropriateness of the “one size fits all” approach, and the necessity of tailoring the testing strategy according to the specific drug and specific pathology (46).

The negative predictive value of skin tests is generally low. This may be partly due to the fact that physiologic metabolites rather than the active drug itself is responsible for the reaction and because many drugs are haptens, which have to be conjugated with a carrier protein before becoming an allergen. Thus, a negative skin test to a drug alone is unreliable for ruling out drug allergy. In the case of a negative skin test, one should consider proceeding to more hazardous drug provocation tests after carefully evaluating the risks and the benefits in the specific patient. On the contrary, even when a proper technique and proper drug material are employed, a positive skin test result does normally indicate the diagnosis. The positive predictive value of a skin test tends to be high, provided that a sufficient number of controls have been tested negative with exactly the same methodology (46).

Skin tests, such as patch, prick, and intracutaneous tests are the most readily available form of allergy testing for physicians, but often do not yield positive reactions, even in patients with well documented histories. Provocation tests i.e. intentional diagnostic drug rechallenge are considered to be the gold standard, but they are not well accepted by patients, because of the risk of severe reactions and are therefore restricted to certain specialist centres with resuscitative equipment. Moreover, for delayed reactions provocation tests are not standardized and a single dose may exclude an IgE-mediated reaction, but not a delayed reaction, which may appear after a higher dose and longer treatment. (46)

A SPT is done by pricking the skin percutaneously with a prick needle through an allergen solution. It is the safest and easiest test, but only moderately sensitive for immediate drug reactions. An intra-dermal test is accomplished by injecting 0.02–0.05 ml of an allergen intra-dermally, raising a small bleb measuring 3 mm in diameter. The intra-dermal test is more sensitive than the SPT, but also carries a higher risk for inducing an irritative, falsely positive reaction and might even lead to an anaphylactic reaction in IgE-dependent reactions. Certain drugs have to be discontinued prior to skin testing (antihistamines, glucocorticoids). The

patient should be free of infectious diseases, fever or inflammatory reactions at the time of testing, unless the skin test is urgently needed. The intake of β-adrenergic blocking agents should be discontinued (usually for 48 h,) according to their half-life of elimination, if the drug to be tested had induced an anaphylactic reaction, as these drugs may interfere with treatment of a possible systemic reaction elicited by the skin test. SPT should be performed on the volar aspect of the forearm. If this is negative after 15–20 min, an intra-dermal test can be performed on the volar forearm, although other regions can be tested (however, there is no comparison for drug allergens). The pain of intra-dermal tests may limit their use in young children.

Normally these tests are well tolerated, but in highly IgE sensitized patients generalized symptoms (urticaria and anaphylaxis) might appear.

Readings should be taken after 15–20 min if immediate reactions are analysed, and after 24 and 72 h for evaluation of non-immediate (late) reactions. In selected cases, additional readings (e.g., after 96 h) are sometimes recommended, as time intervals between testing and positive test reactions may vary. Immediate reactions are documented by measuring the mean diameter of the wheal (and erythema) of the test preparations and the negative control directly after the injection and after 15–20 min. In order to compare the results, a morphological score should be applied as well, enabling a later comparison of different scoring systems. The preferred documentation manner is outlining the size of the injected area and of the reaction at 15–20 min on a translucent cellophane tape. A body of experience has been gained using skin tests in small-molecule drugs, while specific recommendation for biologicals are lacking.

Even within the field of small molecule drugs, a certain level of extrapolation is performed from the most commonly performed tests, such as penicillin tests, and the principles well established for penicillin are applied to skin testing with other drugs. As a criterion for positivity, it is current recommended to employ the criteria used in the diagnosis of penicillin allergy. Reactions are considered positive when the size of the initial wheal increases by 3 mm or greater in diameter after 15–20 min and is associated with a flare (46). Complicating the evaluation is, not only the variability between individual drugs, be they small molecule or biological, but also the multiple possible reactions a drug can cause. Even for one same drug, it is possible that different mechanism may be involved and that the same drug demonstrates different types of immune reactions (47).

In addition to immediate reactions, late reactions, such as delayed or late-phase reactions, should always be examined. They are documented by the diameter of erythema, papulation/infiltrate and morphological description, such as erythematous swelling, erythematous infiltrate, erythema only, eczema with papulation and/or vesicles. Any infiltrated erythema is considered to represent a positive reaction (46).

There is a consensus of opinion that skin tests should be performed after a time interval which allows resolution of clinical symptoms, clearance from the circulation of the incriminated drugs and anti-allergic medications. However, it is not known whether the reactivity might be higher (e.g., cellular hyper-reactivity) or lower (e.g., initial histamine depletion of mast cells or tolerance) if skin tests are performed directly after the reaction (within the next few days). It is also not known to what extent the sensitization to a drug decreases over time. Thus, many groups carry out tests after some minimal time interval of, for example, three weeks, but not after more than three months, if possible (46). This testing strategy, however, appears to be based on common practice and common sense, rather than evidence-based. In particular, this has not been evaluated based on clinical trials.

3.2.1.1 Systemic reactions from skin testing

There are some patients experiencing systemic reactions after skin testing. Patients who had a life-threatening drug hypersensitivity are at risk, even if there is a long time interval between the drug hypersensitivity and skin testing (51). Even fatal outcomes were reported, ass presented in a review published in 1987 (52). However, it is argued that these events were associated with biologic products that are no longer used, such as horse serum-derived tetanus or dyphtheria toxins or pneumococcal antiserum. In the last thirty years the occurrence of systemic reactions, at least with SPT for inhalant allergens extracts, has decreased dramatically. The recent surveys suggest that the overall risk of inducing anaphylactic reactions by SPT is less than 0.02 %, whereas intra-dermal test is more likely to induce systemic reactions. (53) Given the lower specificity and increased risks, intra-dermal test is no longer recommended as first-choice, but for selected diagnostic procedures (49).

Due to the rarity of severe reactions following skin tests, it is difficult to clearly identify all the possible risk factors in the general population, but some basic recommendations can be suggested. Case-control studies gathering data from different centres are needed to evaluate precisely the exact risk factors. In high-risk patients a risk-benefit analysis has to be done: is

the skin test necessary? Are all precautions taken in case of some reactions occur? The risk-benefit analysis has to be made in regard to the clinical reaction, the possibilities of treatment for a possible adverse reactions, the risk for the patient and the importance of the drug. If ever possible, pregnant women should not been tested. The drug should initially be tested with a higher dilution of the test preparations. The next concentration step has to be applied only if the higher dilution has yielded a negative result. In severe, non-immediate reactions it has to be considered to extend the time interval between tests and not to perform intra-dermal test with the highest concentration before performing patch tests (46).

3.2.1.2 Interpretation of skin-test results

Reliable skin test procedures for the diagnosis of drug hypersensitivity are generally missing and test concentrations are unknown or poorly validated for most drugs. For drugs suspected of causing severe reactions or where literature/experience is lacking, skin tests should use nonirritant concentrations of the drug. This can be established using different dilutions of increasing drug concentration (48).

In a large number of patients presenting Hypersensitivity reaction (HSR), no positive findings on either in vivo or in vitro tests is demonstrated. This may be either due to the lack of adequate test reagents or procedures, or may indicate a non-immune pathological mechanism. The negative predictive value of skin tests is generally low. This may be partly due to the fact that physiologic metabolites rather than the active drug itself is responsible for the reaction and because many drugs are haptens, which have to be conjugated with a carrier protein before becoming an allergen. Thus, a negative skin test to a drug alone is unreliable for ruling out drug allergy. In the case of a negative skin test, one should consider proceeding to more hazardous drug provocation tests after carefully evaluating the risks and the benefits in the specific patient (46).

On the contrary, even when a proper technique and proper drug material are employed, a positive skin test result does normally indicate the diagnosis. The positive predictive value of a skin test tends to be high, provided that a sufficient number of controls have been tested negative with exactly the same methodology (46).

The effect of concomitant drugs should s be taken in consideration in the interpretation of the results. Most significant are drugs used for systemic immunosuppression. The effect of

systemic corticosteroid therapy on allergic patch test reactions has been researched. A recent randomized, double blind study involved patch testing individuals with a known nickel allergy to a nickel sulphate dilution series, both during treatment with a 20 mg daily dose of prednisolone and with placebo. Twenty milligrams of oral prednisolone significantly decreased the total number of positive nickel patch tests, increased the threshold concentration for eliciting reactions, and shifted the degree of reactivity towards weaker reactions (54).

Patients with suspected allergies but taking immunosuppressive agents may not always be investigated due to the assumption that positive results would be suppressed. Many patients are heavily dependent on their drugs to control their underlying condition and stopping them before skin tests might be unethical or may lead to a disease flare making testing impossible. There are only few patients in this group and the clinical question remains whether they can be reliably tested or not. A small recent series showed that positive reactions can be seen in patients taking azathioprine, ciclosporin, methotrexate, mycophenolate mofetil, tacrolimus, infliximab, adalimumab, and etanercept (55). The relevance of reactions in this cohort of patients were varied, with some being significant to their presentation and others being of old or uncertain relevance. This study could not, however, shed light on what degree some allergic reactions may have been suppressed by particular immunomodulating drugs.

Importantly, in any situation where the mechanism of ADR is unknown a negative result is unreliable.

3.2.1.3 Skin testing with biopharmaceuticals

The experience with skin testing with small molecule drugs is extensive. It has generally focussed on drugs known to induce anaphylactic reactions such as beta-lactam antibiotics, but also a range of other drugs. The use of skin tests for diagnosis of biopharmaceutical-induced immunogenicity is more recent. There are some notable examples of successful use (56). Skin testing has been successfully used alone or in combination with anti-drug antibody assessment. It has been found useful in predicting serious immune reactions and in assessing the risk and need for desensitisation.

Specific characteristic of biopharmaceutical is that they are essentially all administered parenterally: generally either intravenously or subcutaneously. Every drug administration is therefore an equivalent to a skin test and characteristics of injection site reactions can be

correlated with systemic reactions. As for small molecule drugs, irritative and immune-mediated injection site reactions may be distinguished. The formation of anti-drug antibodies (ADAs) may promote immune-mediated injection site reactions that likely represent either anaphylactic type I reactions, or cutaneous Arthus-like type III reactions according to the Coombs and Gell classification (57). A differentiation between these reactions by clinical course and skin testing may help to decide if treatment should be stopped to avoid the development of more severe ADRs if injection site reactions occur.

The methodology does not vary significantly and the principles tests for small molecule drugs may be applied. With all the limitations discussed above, skin testing may and should be considered as a part of a developmental RMP for biopharmaceuticals in pre-authorization phase. European regulations i.e. the EMA Guideline on Immunogenicity Assessment of Biotechnology-Derived. Therapeutic Proteins suggests that any test which is found useful can be applied i.e. that ongoing consideration should be given to the use of emerging technologies (novel in vivo, in vitro and in silico models) (58). With the advances in standardization of testing, even the old traditional methods such as skin testing may be suitable and useful for modern drugs.

Understandably, skin testing may not always be appropriate, or may not be necessary in for low-risk drugs. On the other hand, the ease of use in the sense of ready availability makes it an appropriate test to consider. As always, the standardization and validation in sufficient number of samples is required for any test. This is an additional reason why a structured and planned testing strategy may be recommended for a large number of drugs in development. The use of skin tests in post-authorization phase can equally be considered as part of post-authorization RMPs or as part of routine clinical use. Of course, depending on the particular circumstance and assessment procedure-associated risk, patient inconvenience and cost versus benefit in terms of adverse reaction risk reduction.

3.2.2 In-vitro tests

In vitro tests would be a safe procedure for patients, avoiding possible disadvantages of in-vivo and provocation tests: new or recall sensitizations to the drug and risk of severe adverse reactions. Moreover, they provide deeper insight into the pathomechanisms involved in drug

hypersensiticity and allow simultaneous assessment of immune responses to multiple drugs. Nevertheless, it is necessary to be aware of the limitations of these tests: they are partly still research tools, standardization is not done for each drug with each test, as exposed controls are missing; They are useful only for certain type of drug allergies (not class II/III reactions according to Gell and Coombs classification) and reflect a sensitization, which is just a risk factor for a symptomatic immune reaction after re-exposure to the drug. However, similar limitations apply to skin tests and provocation tests (59).

3.2.2.1 Antibody determination

The incidence and magnitude of antibody formation depend on a balance of 'foreignness' and the tolerance to the protein. Immune responses to protein drugs that are foreign ('non-self') proteins, or contain portions of a foreign protein, resemble immune responses to vaccines: in most cases, neutralizing antibodies appear that bind to the active site of the biologic and inhibit ('neutralize') its potency by preventing target binding. Protein products that are foreign originate from, or are expressed from, bacteria, plants and nonhuman mammalian systems, such as streptokinase, staphylokinase and the mAb OKT3 (Orthoclone; Ortho Biotech, Bridgewater, NJ). Neutralizing antibodies to such products could also bind to an unrelated site and hinder activity by inducing conformational change. Non-neutralizing antibodies bind to sites on the drug molecule without affecting target binding. Non-neutralizing antibodies are often incorrectly referred to as 'binding antibodies'; but all ADAs (including neutralizing antibodies) are inherently binding antibodies. Although non-neutralizing antibodies do not abolish target binding, they can lower drug bioavailability by increasing the rate of clearance, resulting in an outcome (lowered drug efficacy) that is clinically similar to that observed with neutralizing antibodies. Thus, non-neutralizing antibodies that lower pharmacokinetic parameters are sometimes considered 'clinically neutralizing', but such ADAs are better classified as 'clearing antibodies' (60).

Immune responses to drugs that are structurally identical to human proteins ('self ') are induced by a different mechanism that is based on breaking immune tolerance. How tolerance is induced or broken is not completely understood, but it has been observed that the repetitive administration of proteins and the dose level affect it. Breaking tolerance led to the generation of ADAs against human IFNs, interleukin 2, GM-CSF, erythropoietin and thrombopoietin. It can also occur when a protein is denatured or modified, creating a new antigenic determinant (for example, fusion proteins), when a contaminant is introduced during by

formulation changes (for example, erythropoietin) or when a human protein is given along with a potent adjuvant to enhance its immunogenicity (for example, tumor antigens). In most such instances, patients initially produced ADAs with undetectable neutralizing ability but ultimately developed detectable neutralizing antibodies (61).

Because most therapeutic mAbs developed today are human or humanized, the most likely target for ADAs are the hypervariable or complementarity-determining regions (CDRs) that provide the majority of binding contacts. The immunogenicity of CDRs often leads to the production of neutralizing antibodies, but non-neutralizing antibodies to these sequences or other parts of the mAb may also be elicited.

Presumably, the incidence of ADAs and neutralizing antibodies within the drug development phase predicts anti-drug immune response incidences in clinical practice (post-marketing). Yet the incidence of ADAs and neutralizing antibodies in controlled clinical studies may not reliably estimate that seen during the post-approval stage, with larger numbers of exposed subjects, more concomitant medications, repeated drug re-exposures and reduced patient treatment compliance. Nonetheless, measuring drug-induced ADAs during drug development is important. To do this, and to provide context to immunogenicity data, it is vital to understand the test methods used and their caveats.

Two types of platform technologies exist:

(i) immunoreactivity assays such as radioimmunoassay, surface plasmon resonance or enzyme-based solid-phase immunoassays, to detect ADAs; and
(ii) functional cell-based bioassays or target binding (receptor recognition) inhibition-based immunoassays for the characterization of the neutralizing antibodies subset of ADAs.

Both assay types can be used together for the complete characterization of the antibody response against a drug molecule. The ADA immunoreactivity assays can be further divided into three subtypes that include:

- first, a sensitive screening immunoassay to identify samples potentially positive for ADAs;
- second, a specificity confirmation immunoassay that eliminates false positives; and

- third, an immunoassay to obtain a relative measure or titre of the ADA concentration in serum.

When appropriate, cell-based neutralizing antibody bioassays or target binding inhibition–based neutralizing antibody immunoassays are also conducted to characterize the neutralizing ability of the ADAs. Samples can also be characterized for ADA isotyping by immunoassay, but the value of this approach may be limited. Sensitive detection assays combined with appropriate characterization of the ADAs can provide helpful information directly related to patient safety and treatment as well as overall understanding of the humoral immune response to therapeutic proteins.

Whereas the development and validation of sensitive and reproducible methods should be the goal for ADA bioanalysis, at present, standardized assays are not available and reference standards are rarely available, which make it difficult to compare results obtained from different laboratories and different studies. The incidence of ADA may also be limited by the assay method used—for example, low-affinity ADAs by surface plasmon resonance versus immunoassays that use multiple wash steps. Similarly, some additional limitations of ADA test methods must also be understood.

First, the ‘sensitivity’ of a method is dependent on the affinity of the positive control used to characterize it, making it inappropriate to compare across test methods employing different positive controls, and even more so for ADA test methods of different products.

Second, the therapeutic protein often interferes with ADA assays, and this ‘drug tolerance limit’ is generally characterized; in such instances, it is a common malpractice to apply the drug tolerance limit in deciding a subject’s ADA status (that is, when ADAs are undetectable and the drug level in that sample is below the drug tolerance limit, it is reported as ADA negative). Because the tolerance limit, like sensitivity, is dependent on the affinity of the individual ADA and drug, it cannot be represented by the tolerance limit of the assay positive control. Thus, drug tolerance limits should not be used in determining ADA status; instead, study designs should allow for the collection of data from at least one time point where drug has been fully cleared from the circulation. The assessment of treatment-emergent ADAs should be made per individual subject and should use a prospective decision tree to characterize the subject appropriately.

Third, neutralizing antibody assays—whether cell-based bioassays or target binding inhibition–based immunoassays—are also limited by sensitivity, and lack of neutralizing activity in these assays does not confirm that the ADA is a non-neutralizing antibody (60).

For all these reasons, it is inappropriate to compare ADA incidence rates between different drug products, and certainly between products from different companies. In fact, the US Food and Drug Administration (FDA) has required that biological product package inserts explicitly state that comparisons can be misleading.

3.2.2.2 In vitro diagnostic tests of cell-mediated immunity

As in vitro tests rely on the presence of drug-reactive immune cells in blood of drug-sensitised patients, persistence and frequency of these cells has a crucial impact on in vitro diagnosis of drug hypersensitivity. Beeler et al (15) demonstrated that 1:250 – 1:10 000 of T cells in the peripheral blood of patients in the remission react to the relevant drug. This study also showed that T cells can persist as memory cells in peripheral blood of drug-allergic patients for up to 12 years after disease outcome. On the contrary some patients can lose reactivity 1–3 years after the original treatment with drugs that caused hypersensitivity reaction (62). At present, it is impossible to predict how long the reactivity of drug-specific T cells in an individual patient will persist.

In vitro tests are normally done during remission of disease, because peripheral blood mononuclear cells (PBMC) obtained ex vivo from acute drug-allergic patients are strongly activated. This could lead to high background proliferation and difficulties in detecting an enhanced proliferation after drug stimulation. The time interval between acute stage and test performance allows washing out the incriminated drugs and any anti allergic drugs, which may suppress the immune response in vitro. Thus, according to common opinion, in vitro tests should be performed after a minimal time interval of 3 weeks after the DHR. An analysis in the first 6 months or minimally first year is recommended, but later tests may still be positive due to the long-persisting T cells specific for the drug (59).

In Vitro Diagnostic Tests of Cell-Mediated Immunity: Basophil activation test

The Basophil activation test (BAT) detects specific markers that are expressed on the surfaces of blood basophils after their activation by incubation with the responsible drug.

In a first step the basis of these assays is the identification of basophils by specific fluorescent antibodies such as anti-IgE, anti-CD123 (IL-3 receptor) and anti-HLA-DR or anti-CCR3, and in a second step the demonstration of certain membrane phenotypes that appear after exposure to allergen. Most studies in the literature make reference to the expression of CD63 or CD203c on basophils after their in vitro activation. CD63 is a tetraspan, 53-kDa granular protein that is expressed not only on basophil granules but also on monocytes, macrophages and platelets. The expression of this marker correlates with degranulation and histamine release, which makes it an ideal marker of basophil activationCD203c antibodies recognize a type-II transmembrane protein which is increased on the surface after activation (68).

With the help of receiver-operated curves (ROC; optimal sensitivity versus specificity), the determination of a positivity cut-off must be made for each allergen in order to evaluate the results. It is important to consider the factors affecting the positive as well as the negative control. Natural exposure in vivo to the allergen can cause high basal activation (affecting negative control), for example in a pollen- allergic patient studied during the pollen season. Most studies use mono- or polyclonal anti-IgE as positive control (68).

Successful performance of BAT depends on the drug. Results obtained for one drug will not necessarily apply to another. Timing of testing, storage of blood following sampling, concentrations of antigen, positive and negative controls, selecting of markers for activation are just some among a multitude of factors to be considered and standardized for each drug. Notable success has been documented for several common antigenic drugs as well as other allergens. (69) However, the usefulness of this test has not been demonstrated for biopharmaceuticals. No notable publications are available in the public domain and the reasons for this absence of information is subject to individual interpretations.

BAT was evaluated in correlation with outcomes of rechallenge in children with IgE-mediated cow's milk allergy. Oral challenge was compared to the BAT, the specific IgE and SPT results. The percentage of activated basophils in patients with a positive challenge was significantly higher than that of patients with a negative challenge, and was well correlated

with the eliciting dose of cow's milk. The BAT had an efficiency of 90%, a sensitivity of 91%, a specificity of 90%, and positive and negative predictive values of 81% and 96% in detecting persistently allergic patients. These scores were higher than those obtained with SPT and IgE values, whichever positivity cut-point was chosen. Referring to a decisional algorithm combining BAT, specific IgE and SPT allowed the correct identification of 94% of patients as tolerant or persistently allergic to cow's milk proteins in a cohort (70). This successful basis for a rechallenge decision tree is very close to the subject of this thesis. Even though a drug was not used, a biological protein product was used, albeit in a manner unlikely to be used for therapeutic purpose with biopharmaceuticals and in a very specific condition.

In Vitro Diagnostic Tests of Cell-Mediated Immunity: Lymphocyte transformation test

Lymphocyte transformation test (LTT) is currently the most widely used test for diagnosis of T cell-mediated drug hypersensitivity. The LTT relies on the activation and proliferation of T cells after stimulation with the specific drug under *in vitro* conditions. This concept of the LTT has been confirmed by the generation of drug-specific T cell clones (63).

In the LTT, PBMC are obtained from a drug-sensitized patient and cultured in the presence of the suspected drug. Drug-specific T lymphocytes undergo blastogenesis and generate cytokines such as IL-2, followed by a proliferative response that is measured by the incorporation of ^{3}H-thymidine during DNA synthesis after 6 days of culture. Although ^{3}H-thymidine uptake is measured in counts per minute, results of the LTT are given as stimulation index, which is the ratio of cell proliferation with antigen divided by the background proliferation, without drug.

The result of T cell activation is secretion of cytokines or cytotoxic mediators, and proliferation. Activation starts within a few minutes after triggering of the TCR by a specific drug antigen presented by major histocompatibility complex (MHC) class I or II, and is followed by increase of intracellular Ca21, as well as the activation of early genes of antigen recognition, including critical transcription factors (nuclear factor of activated T cells, activator protein-1, and nuclear factor-kB). Within hours a number of genes encoding for various cytokines (IL-2, 3, 4, 5, and 6, IFN-g, TGF-b) and early activation markers (CD40L, CD69, CD25, CD71) are expressed (64). Around 1–2 days after T cell activation, IL-2

induces the proliferation of activated T cells, which goes along with additional gene expression and DNA synthesis. Approximately 3–5 days after activation, T cells enter the phase of functional differentiation, which drives to production of distinct cytokine patterns which determine the effector functions of T lymphocytes. In vitro tests may grasp distinct parameters of this differentiation, ranging from surface marker up-regulation to cytokine production, proliferation, and cytotoxicity (59).

It should be taken into consideration that treatment with corticosteroids or other immunosuppressive drugs may influence the test results by suppressing the proliferation *in vitro*. On the other hand, some drugs (vancomycin, possibly paracetamol, as well as certain radio-contrast media and non-steroidal anti-inflammatory drugs) may elicit slightly enhanced proliferation even in non-sensitized individuals (63). Stimulation index value is not associated with the severity of clinical symptoms. The LTT in patients with MPE might reveal strong T cell proliferation and higher stimulation index values than the LTT performed in patients with severe forms of drug hypersensitivity, such as SJS or TEN. In fact, the clinical severity of a drug hypersensitivity reaction seems to be related rather to the effector function of reacting cells, than to the high frequency of these cells in the peripheral blood detected by the LTT.

Several studies performed to date indicated that the general sensitivity of the LTT in well-defined ADR may lie between 60% and 70%. It depends on the drug, the type of reaction and is superior to skin testing for non-immediate type reactions (59). In the prominent retrospective study on the LTT accuracy, 923 patients were classified according to the imputability of ADRs (63). In patients classified as definitely drug allergic the LTT yield a sensitivity of 78%, while patients with lower clinical likelihood of drug hypersensitivity had a respectively lower incidence of a positive LTT (63). The specificity of the LTT was in the range of 85–100% in different studies and, similarly to sensitivity, may depend on the individual drug (59). On the one hand, the LTT has some limitations: it requires experience with cellular techniques; involves radioactivity, and thus certain expensive equipment, it is rather cumbersome as it relies on a 6-day sterile culture, and, most importantly, negative results cannot exclude drug hypersensitivity. On the other hand the test is applicable with many different drugs and types of immunologic ADRs. Positive LTT may not only support the diagnosis of drug allergy, but can also pinpoint the responsible agent, in case patient has taken several drugs (59).

Beta tryptase

The release of β- A serum mast cell β-tryptase test could be helpful if other specific tests, such as drug specific IgE assay is not available. The release of β-tryptase from the secretory granules is a characteristic feature of mast cell degranulation. While its biological function has not been fully clarified, mast cell β-tryptase has an important role in inflammation and serves as a marker of mast cell activation (73).

After anaphylaxis, mast cell granules release tryptase; measurable amounts are found in blood, generally within 30 to 60 minutes. The levels decline under first-order kinetics with half-life of approximately 2 hours. By comparison, histamine is cleared from blood within minutes (74).

B-tryptase is useful in identifying mast cell mediated reactions. In that way it can help in differentiating between anaphylactic reactions and infusion reaction due to cytokine release. However, it cannot differentiate between immune mediated anaphylaxis and non-immune i.e. anaphylactoid reactions. In the latter case, tryptase is equally elevated, but due to direct mast cell activation independent of IgE.

3.2.2.3 Alternatives to the conventional antibody determination strategies

Although regulatory authorities recommend cell-based assays for detection of neutralizing antibodies, such assays are difficult to standardize, and ill adapted to high-throughput analysis. Their use has generally been limited to clinical trials and research laboratories, while routine clinical application has not been feasible. The logical alternative would be to develop antibody assays which are less cumbersome, even if they do not meet the regulatory standards required for pre-marketing drug evaluation (or at least, if they do not meet the current standards). Such assays, if adequately validated, may still be practical for routine clinical application.

The current regulatory standard is a three-step assay battery, as described in the EMA Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (58). The down-side of such a battery of test is clearly the time and resource required. A proposed solution is a one-step cell-based assay that allows both drug activity and drug NAbs to be quantified rapidly and with a high degree of precision simply be adding reporter cells to a sample. In the assay described by Lallemand and colleagues in 2010, the reporter cells have

been engineered to detect both drug concentrations of interferon alfa in the first step, and inhibition by Nabs in the second step of the assay (75). The reporter cells express firefly luciferase under the control of a drug-responsive promoter. Subsequently, they also express the drug of interest (interferon alfa in the published example), the production of which is normalized relative to the expression of Renilla luciferase transcribed from a common doxycycline-inducible promoter. Residual drug levels present in a sample are first quantified by determination of firefly luciferase expression, autocrine drug synthesis is then induced, and neutralizing antibody activity is quantified from the difference in the ratio of firefly luciferase / Renilla luciferase expression in the presence or absence of the sample. Since assay results are normalized relative to the expression of an internal standard, results are independent of cell number or differences in cell viability thus affording a high degree of assay precision and reducing serum matrix effects to a minimum. This unique assay platform is suited for high-throughput analysis, and according to the authors, it is applicable to most biopharmaceuticals.

3.3 *Product quality assessment*

Biotechnology products, in many cases recombinant proteins, are derived from complex expression/production systems that often involve genetically modified host cells (bacteria, yeast, or mammalian) and complex growth/fermentation media. The ensuing purification steps may be insufficient to completely eliminate impurities such as DNA, host cell proteins, or endotoxins from the product. As a result, biologics may contain low levels of host cell or process derived impurities. These impurities can potentially stimulate the innate immune system via a host of pattern recognition receptors and foster the development of an immune response to the product.

In case of therapeutic vaccines it is advantageous to boost the immune response by the addition of vaccine adjuvants. In all other cases, particularly biopharmaceuticals, it is imperative to strictly control unwanted immunogenicity caused by the presence of impurities, whether they are degradation products or process derived impurities.

Non-clinical studies on murine splenocytes demonstrated that the effect of impurities was dose dependent and synergistic, as levels of impurities that individually induced no or very low levels of cytokine release by, elicited polyclonal B cell activation with increased antigen-

specific immunoglobulin and pro-inflammatory/Th1 cytokine output as well as up-regulation of co-stimulatory molecules on the cell surface of antigen presenting cells. This synergistic effect was then confirmed in vivo, as studies showed that the combination of impurities, which do not induce an immune response when present individually, were sufficient to promote the immunogenicity of proteins and contribute to a clinically relevant break in tolerance to self. (76)

On the clinical level, the effect of product quality has been illustrated by the example of pure red cell aplasia with the use of eythropoetin products (10). This case was reported in the literature as a rare and dramatic adverse reaction. However, many additional cases may remain unpublished and outside of the public domain. The true incidence of quality-related safety concerns this remains unknown.

3.4 Further challenges: biosimilars

Notably, all mAbs covered by this analysis are originator products. Biosimilars, unlike small molecule drugs, are not generic medicines, as they are not identical to their respective reference biologic drugs. Preclinical and clinical studies must be carried out to demonstrate that biosimilars and their reference biologic drugs have comparable efficacy and safety. A patient switched between two biologic drugs might develop anti-drug antibodies (caused by small differences in post-translational modifications of the proteins and/ or product/process-related impurities), which could compromise efficacy and safety. Marketing of biosimilar versions of these complex proteins poses further challenges to safety assessment (77).

For some biologics, there are multiple innovator versions that are safe, effective, and non-identical; therefore, some differences between products are not critical. Innovators also make manufacturing changes with minimal or no supporting clinical data. The studies required to determine whether a product undergoing a manufacturing change is equivalent to the product prior to the change will depend on the stage at which the changes are introduced, the impact or potential impact on the product, analytical limitations, and the link between the quality criteria and possible implications on safety and efficacy. As a result, the comparability exercise undertaken by innovators after a manufacturing change can be used as a model for biosimilar comparability, and adapted international guidance (especially from ICH including

Q5E and Q6B) for this purpose (78, 79).

While Q5E applies only to changes within one manufacturer, much of the guidance is relevant to biosimilars. One of the general principles of the guideline is that "the demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon the safety or efficacy of the drug product"; therefore, the previously derived clinical data are still relevant. If comparability is not established, more extensive clinical trials are usually requested (80). Predictability of reactions to biosimilar biopharmaceuticals should take into account all the relevant specificities of individual biosimilar products.

4 OBJECTIVES

The overall objective of this thesis is to identify and assess the factors determining the safety of biopharmaceuticals, taking into account all available non-clinical and clinical methods for evaluation. Immunogenicity is the most specific safety concern of biopharmaceuticals and therefore the focus of this thesis.

- The specific primary objective is to assess to what extent serious adverse reactions are predictable and to identify the available methods for reliable evaluation of immunological adverse reactions.
- Analysis of time to detection of adverse reaction is also regarded as an individual objective, since early detection is of major importance for public health.
- Rechallenge of patients who developed an immune-mediated reaction is given special attention as a secondary objective. It is of particular importance for biopharmaceuticals as it may lead to a more severe reaction following subsequent administration.

The starting point is purely scientific evaluation of predictability based on the available literature sources including regulatory databases and scientific publications. However, regulatory aspects of benefit-risk assessment and risk management planning within the pharmaceutical development and product life cycle management are considered.

5 METHODS

Methods applied were twofold:

- The initial step consisted of regulatory authority medical product database search for adverse drug reactions to biopharmaceuticals. The safety profile known at the time of marketing authorization application was compared to the safety profile emerging post-authorization. The search was performed on the US FDA Safety Information and Adverse Event Reporting Program (FDA Medwatch). The search focused on safety alerts for monoclonal antibody therapeutics. This search included all reported safety alerts whether they were immunological in nature or not.
- The second step consisted of Kaplan Meier analysis of time to Medwatch safety alert. This analysis was based on the data on safety alerts collected as part of the initial step. Kaplan-Meier estimate is one of the best options to be used to measure the fraction of subjects living for a certain amount of time after treatment. In the current analysis, the time to safety alert was used as time to event i.e. "survival", which is commonly measured using Kaplan-Meier analysis.

This analysis focusses on therapeutic monoclonal antibodies, and in particular, it focusses on mAbs on US market. Alerts issued in various countries and various regions are not expected to show no major differences, as they are all a reflection of global signal management and benefit-risk evaluation. Medwatch safety alerts were preferentially selected over the EMA alerts since the website lists alerts and full history of approval letters and previous (non-current) product labelling. The choice of the regulatory authority as the source of information is not expected to significantly affect the results.

5.1 Regulatory authority medical product database search

US FDA Medwatch safety alert as posted on MedWatch Medical Product Safety Information web site (http://www.fda.gov/Safety/MedWatch/SafetyInformation/default.htm) was taken as search trigger. The site provides information for 10 years i.e. January 2000 – December 2009 inclusive, when most mAbs have been marketed. Prior alerts were identified from publications. Adverse reaction terms in the alert message were cross-checked with the initial

product label at the time of approval from Drugs@FDA site: (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm). If the label did not provide sufficient data, the FDA medical or clinical review was consulted.

Alerts were assigned to one of the two categories:

- **Observed:** An alert was considered observed when increased frequency, severity, or other new properties were reported for essentially similar previously identified suspected adverse reactions.
- **Not observed**: This categorisation was applied to reactions not described in the product label. It does not refer to events which may have been suspected based on mechanism of action or chemical structure. This novel definition is based on objective clinical findings. It does not account for pharmacological-toxicological assessment of what may have been predicted based on chemical structure or antibody target.

The so-called "observed" alerts may be considered anticipated, expected, or predictable. Whatever terminology is used, they describe adverse reactions for which there was clinical evidence or suspîcion. The findings were not necessarily defined as identified or potential risks, but were observed. Alerts for product quality issues as well as medication errors are not taken into account without reported associated adverse reactions. Such product quality related alerts are due to product manufacturing whereas medication errors may be a result of product packaging, naming or instruction for use i.e. they are not a result of the medicinal product itself. Where adverse reaction is reported, this is taken into account irrespective of the potential association with manufacturing/quality issues or medication errors.

If an alert message referred to several reactions identified simultaneously, they were analysed and counted as separate. Alerts referring to a therapeutic class (e.g. TNF-α blockers) were analysed with respect to each individual drug. If an alert appeared more than once, e.g. early communication due to on-going review, reminder of previous alerts, or alert for an individual medication and additionally for a group, each alert counted as a separate occurrence.

Simple summary statistics was performed. Both summary statistics as well as graphical representation (pie chart) were performed using Microsoft Excel, Microsoft Corporation, Redmond, WA 98052-7329, USA.

5.2 Kaplan-Meier analysis

The Kaplan-Meier estimate is the simplest way of computing the survival over time in spite of difficulties associated with subjects or situations. The survival curve can be created assuming various situations. It involves computing of probabilities of occurrence of event at a certain point of time and multiplying these successive probabilities by any earlier computed probabilities to get the final estimate. This can be calculated for two groups of subjects and also their statistical difference in the survivals (82).

Kaplan and Meier (83) proposed a non-parametric estimate which specifies a discrete distribution. All the probability is concentrated at a finite number of points, or else (for a large sample) an actuarial approximation thereto, giving the probability in each of a number of successive intervals. The method also considers how such estimates are affected when some of the lifetimes are unavailable (censored) because the corresponding items have been lost to observation, or their lifetimes are still in progress when the data are analysed.

The analysis uses time to event, commonly referred to as "survival", even when the event of interest is not a fatal outcome but another event. The equivalent to survival in this analysis is the time to Medwatch safety alert.

The Kaplan Meier analysis was clearly not designed to treat the type of data under evaluation in this thesis. In fact, it was primarily designed to treat the data on clinical or epidemiological observations which follow subjects over long time periods. Its use has been extended outside of its usual application to an unconventional type of data. Several modifications to the original intent and the usual use, of the Kaplan-Meier analysis were implemented for this purpose.

Kaplan-Meier estimate or Kaplan-Meier "survival analysis" is sometimes used to measure time to event, even if this event is not death but another relevant study outcome. This event may be recurrence of tumour, appearance of seizures for patient on anti-epileptic treatment or appearance of any other relevant event, even if the analysis is used in fields other than medicine. Another example of its use is to examine "drug survivorship". This term is used to describe how long patients continue to tolerate a particular drug before either side-effects or lack of efficacy cause them to be switched to some other therapy. An evaluation of the applicability of Kaplan-Meier analysis to drug survivorship in rheumatology studies questioned its validity (84). Kaplan-Meier analysis is used to analyse survival in trials in which not all patients reach the endpoint – this outcome is frequently subject death. However, the initial intent of Edward L. Kaplan and Paul Meier was apparently not purely survival, but

more broadly incomplete observations (83).

The Kaplan-Meier analysis was performed using MedCalc software: Version 12.4.0 -ast modified: January 2, 2013, © 1993-2013 MedCalc Software bvba, MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium. http://www.medcalc.org/

6 RESULTS

6.1 Predictability of serious adverse reaction alerts for monoclonal antibodies

Up until January 2010, inclusive, 36 safety alerts to mAbs were issued containing 61 alert terms (see Table 2). These alerts apply to 17 mAbs, out of 27 authorized by the FDA by the end of the observation period. (81)

According to the assessment criteria described above, 32 out of 61 (i.e. just above a half of the alert terms) were assessed as observed (see
Figure 5 and Table 2). Two antibodies were withdrawn from the market during the observation period: Technetium (99m Tc) fanolesomab and efalizumab.

Many unobserved reactions could have been predicted. Listing just several examples:

– Immunogenicity and anti-drug antibodies, but no hypersensitivity reactions, were detected in pre-approval trials for adalimumab, basiliximab and daclizumab.

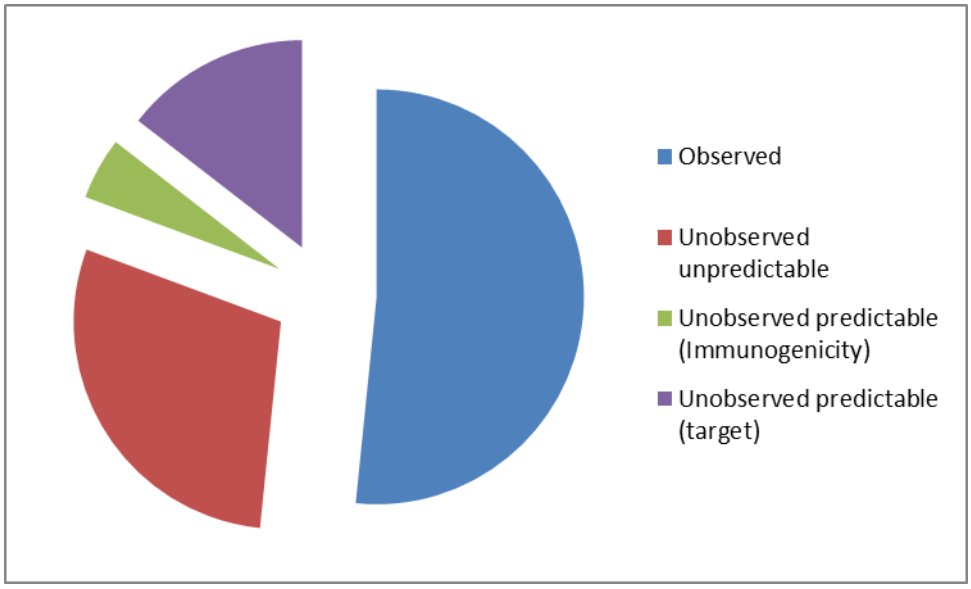
– Bevacizumab alert dated April 2007 described cases of tracheoesophageal fistula. Very similar to gastrointestinal perforations described in initial label (February 2004) this event is apparently a direct extension of bevacizumab's main mechanism of action i.e. binding to vascular endothelial growth factor (VEGF). Arguably difficult to predict, this reaction serves as an example of how much it may be possible to suspect, if not predict, similar ADRs.

– Rituximab's potential for increasing susceptibility to infections was only demonstrated post- approval, even though this was likely based on its mechanism of action (binding to CD 20, human B-lymphocyte- restricted differentiation antigen).

– Increased likelihood of infections as well as likelihood for carcinogenicity for TNF-α blockers could have equally been suspected based on their mechanism of action.

Such "unobserved" but arguably predictable alerts represent an important percentage. There are 3 alerts for immunologic reactions and another 9 arguably predictable based on antibody target. Therefore, 18 out of 61 alerts (29.5%) according to this extended classification remain unobserved and unpredictable. Please see details in Figure 5 and Table 2. Unobserved but predictable alerts are marked by § sign in Table 2.

Figure 5: Adverse reaction predictability based on Medwatch safety alert : taking into account alerts predictable based on structure and target.



On the other hand, many alerts were issued for reactions which were classified as observed:

– An illustrative example of an observed alert is trastuzumab cardiotoxicity. According to the initial Sep- 1998 FDA approved label: "HERCEPTIN administration can result in the development of ventricular dysfunction and congestive heart failure." This warning was based on clinical trials using other cardiotoxic drugs (anthracycline chemo therapy), but results of post-approval trials strengthened the evidence of the causative role of trastuzumab; hence the August 2005 cardiotoxicity alert was classified as observed.

– Immunomodulation by TNF-α inhibitors lead to several alerts for increased rate of infections, in particular opportunistic infections including histoplasmosis and tuberculosis. Such observations are congruent with animal studies showing that TNF is important for granuloma formation (82) and preventing the reactivation of latent tuberculosis (86). Increased rate of some types of infection doesn't necessarily mean increased susceptibility to all infectious pathogens, but some infections may be predictable based on common cellular or humoral response mechanisms. Since an increased infection rate should dictate vigilance towards all infections, we classified these alerts as observed.

Some cases were difficult to describe as clearly observed or not e.g. the Remicade (infliximab) December 2004 alert for severe hepatic reactions, including acute liver failure, jaundice, hepatitis and cholestasis. Hepatic enzymes were elevated in more than 1% and less than 5% during pre-approval clinical trials (according to the initial label US FDA approved in Aug-1998). The FDA clinical review was consulted which noted that analysis of clinical chemistry laboratory evaluations were noteworthy for the changes seen in creatinine and in liver function parameters. Hence, the hepatic reactions were assessed as observed.

Results of the search and evaluation of predictable vs unpredictable Medwatch adverse drug reaction alerts are presented in Table 2. Antibodies for which no alert is reported are not presented in the table.

Table 2: Adverse reaction predictability based on Medwatch safety alert

Name	Target	Alert Date	Alert Message	Observed (Yes/No)
Avastin (bevacizumab)	VEGF	Aug-2004	Arterial thromboembolic events	Y
		Jan-2005	Arterial thromboembolic events.	Y
		Sep-2006	Reversible posterior leukoencephalopathy syndrome	N
			Nasal septum perforation.	N
		April-2007	Tracheoesophageal fistula §	N
		Jul-2008	Microangiopathic hemolytic anemia in combination with sunitinib malate	N
Erbitux (cetuximab)	EGF receptor	Sep-2005	Observation periods following infusion	Y
			Hypomagnesemia	N
Herceptin (trastuzumab)	HER2	May-2000	Hypersensitivity reactions	Y
			Infusion reactions	Y
			Pulmonary reactions	N
		Aug-2005	Cardiotoxicity	Y
Humira (adalimumab)	TNF-α	Nov 2004	Infections with the combined use of anakinra	N
			Hypersensitivity reactions, including anaphylaxis §§	N
			Hematologic events, including pancytopenia and aplastic anemia	Y
Lucentis (ranibizumab)	VEGF-A receptor	Feb 2007	Stroke higher incidence with higher dose	Y
NeutroSpec (Technetium fanolesomab)	CD15	Dec-2005	Cardiopulmonary events (market suspension)	N

Raptiva (efalizumab)	CD11a	Jul-2005	Immune-mediated hemolytic anemia	N
			Thrombocytopenia	Y
			Infections	Y
		Oct-2008	PML, fungal and other opportunistic infections	Y
		Feb-2009	PML (market suspension)	Y
Remicade (infliximab)	TNF-α	Oct-1998	Adverse events due to antibodies	Y
		Oct-2001	Tuberculosis and other opportunistic infections	Y
		Oct-2001	Heart failure	N
		Aug-2004	Lymphoma and other malignancies	Y
		Dec-2004	Hepatic reactions	Y
Rituxan (rituximab)	CD20	Nov-1998	Infusion reactions	Y
		Oct-2004	Hepatitis B virus reactivation §	N
		Dec-2006	PML §	N
		Sep-2008	PML leading to death18 months after the last dose §	N
Simulect (basiliximab)	CD25	Oct-2000	Hypersensitivity reactions, including anaphylaxis §§	N
Synagis (palivizumab)	RSV F protein	Nov-2002	Anaphylaxis	Y
TNF-α blockers (Remicade, Humira,	TNF-α	Jun-1998	Lymphoma and other cancers in children and young adults §	Cimzia: N Humira: Y Remicade: Y

Cimzia, and Simponi (golimumab))		Sep-2008	Histoplasmosis, coccidioidomycosis, blastomycosis and other opportunistic infections	Cimzia: Y Humira: Y Remicade:Y
		May-2009	Histoplasmosis and other invasive fungal infections	Cimzia: Y Humira: Y Remicade: Y
		Aug-2009	Lymphoma and other cancers in children and young adults §	Cimzia: N Remicade: Y Humira: Y Simponi: Y *
Tysabri (natalizumab)	α4β1 and α4β7 integrins	Feb-2005	PML §	N
		Feb-2008	Liver injury	Y
		Aug-2008	PML §	N
		Sep-2009	PML §	N
Xolair (omalizumab)	IgE receptor	Feb-2007	Anaphylaxis	Y
		Jul-2009	Ischemic heart disease	N
			Arrhythmias	N
			Cardiomyopathy and cardiac failure	N
			Pulmonary hypertension	N
			Cerebrovascular disorders	N
			Embolic, thrombotic and thrombophlebitic events	N
Zenapax (daclizumab)	CD25	Aug-2003	Increased mortality	N
			Hypersensitivity reactions §§	N

Zevalin (ibritumomab tiuxetan)	CD20	Oct-2005	Cutaneous or mucocutaneous reactions	Y

Legend:

- * Simponi (golimumab) was approved in Aug-2009, hence alerts for TNF-α inhibitors take Simponi into account only following its marketing approval.
- § Alert is unobserved prior to marketing authorization and unpredictable based on the strict definition of predictability used in the analysis, but predictable based on the antibody target
- §§ Alert is unobserved and unpredictable based on the strict definition of predictability used in the analysis, but predictable based on the antibody structure (immunological reaction).
- Abbreviations: EGF, Epidermal growth factor; HER2, human epidermal growth factor receptor 2; RSV, respiratory syncytial virus; VEGF, vascular endothelial growth factor.
- Table taken with minor modification from Stanulovic et al, 2011 (87).

In addition to the overall presentation of Medwatch safety alerts, a breakdown is also presented based on the origin of the antibody i.e. the species from which the antibody is obtained. The first monoclonal antibodies were produced in mice (substem -o-, yielding the ending -omab). Two such antibodies with reported Medwatch safety alerts are presented in Table 3, below (Technetium fanolesomab and ibritumomab tiuxetan). Both antibodies in the subgroup have a chelator, to which a radioactive isotope is linked.

Table 3: Murine antibody alerts

Name	Target	Alert Date	Alert Message	Observed (Yes/No)
NeutroSpec (Technetium fanolesomab)	CD15	Dec-2005	Cardiopulmonary events (market suspension)	N
Zevalin (ibritumomab tiuxetan)	CD20	Oct-2005	Cutaneous or mucocutaneous reactions	Y

Chimeric antibodies, in which part of the constant region of animal/foreign origin is replaced with the human form, are identified by the substem -xi-. Four such antibodies with reported Medwatch safety alerts are presented in Table 4, below (cetuximab, infliximab, rituximab, basiliximab – with the note that infliximab is presented as alerts reported for itself as well as for its therapeutic group of TNF-α blockers).

Table 4: Chimeric antibody alerts

Name	Target	Alert Date	Alert Message	Observed (Yes/No)
Erbitux (cetuximab)	EGF receptor	Sep-2005	Observation periods following infusion / infusion reactions	Y
			Hypomagnesemia	N
Remicade (infliximab)	TNF-α	Oct-1998	Adverse events due to antibodies	Y
		Oct-2001	Tuberculosis and other opportunistic infections	Y
		Oct-2001	Heart failure	N
		Aug-2004	Lymphoma and other malignancies	Y
		Dec-2004	Hepatic reactions	Y
Rituxan (rituximab)	CD20	Nov-1998	Infusion reactions	Y
		Oct-2004	Hepatitis B virus reactivation	N
		Dec-2006	PML	N
		Sep-2008	PML leading to death18 months after the last dose	N
Simulect (basiliximab)	CD25	Oct-2000	Hypersensitivity reactions, including anaphylaxis	N
TNF-α blockers (Remicade (infliximab))	TNF-α	Jun-2008	Lymphoma and other cancers in children and young adults	Remicade: Y
		Sep-2008	Histoplasmosis, coccidioidomycosis, blastomycosis and other opportunistic infections	Remicade:Y
		May-2009	Histoplasmosis and other invasive fungal infections	Remicade: Y
		Aug-2009	Lymphoma and other cancers in children and young adults	Remicade: Y

Part of the variable regions may also be substituted, in which case the antibody is called humanized and -zu- is used; typically, everything is replaced except the complementarity determining regions (CDRs), the three loops of amino acid sequences at the outside of each variable region that bind to the target structure. Six such antibodies with alerts are presented in Table 5, below (bevacizumab, trastuzumab, ranibizumab, efalizumab, palivizumab, while alerts for certolizumab pegol are presented as therapeutic group alerts for TNF-α blockers).

Table 5: Humanized antibody alerts

Name	Target	Alert Date	Alert Message	Observed (Yes/No)
Avastin (bevacizumab)	VEGF	Aug-2004	Arterial thromboembolic events	Y
		Jan-2005	Arterial thromboembolic events.	Y
		Sep-2006	Reversible posterior leukoencephalopathy syndrome	N
			Nasal septum perforation.	N
		April-2007	Tracheoesophageal fistula	N
		Jul-2008	Microangiopathic hemolytic anemia in combination with sunitinib malate	N
Herceptin (trastuzumab)	HER2	May-2000	Hypersensitivity reactions	Y
			Infusion reactions	Y
			Pulmonary reactions	N
		Aug-2005	Cardiotoxicity	Y
Lucentis (ranibizumab)	VEGF-A receptor	Feb 2007	Stroke higher incidence with higher dose	Y
Raptiva (efalizumab)	CD11a	Jul-2005	Immune-mediated hemolytic anemia	N
			Thrombocytopenia	Y
			Infections	Y

		Oct-2008	PML, fungal and other opportunistic infections	Y
		Feb-2009	PML (market suspension)	Y
Synagis (palivizumab)	RSV F protein	Nov-2002	Anaphylaxis	Y
TNF-α blockers Cimzia (certolizumab pegol)	TNF-α	Jun-2008	Lymphoma and other cancers in children and young adults	Cimzia: N
		Sep-2008	Histoplasmosis, coccidioidomycosis, blastomycosis and other opportunistic infections	Cimzia: Y
		May-2009	Histoplasmosis and other invasive fungal infections	Cimzia: Y
		Aug-2009	Lymphoma and other cancers in children and young adults	Cimzia: N
Tysabri (natalizumab)	α4β1 and α4β7 integrins	Feb-2005	PML	N
		Feb-2008	Liver injury	Y
		Aug-2008	PML	N
		Sep-2009	PML	N
Xolair (omalizumab)	IgE receptor	Feb-2007	Anaphylaxis	Y
		Jul-2009	Ischemic heart disease	N
			Arrhythmias	N
			Cardiomyopathy and cardiac failure	N
			Pulmonary hypertension	N
			Cerebrovascular disorders	N
			Embolic, thrombotic and thrombophlebitic events	N
Zenapax (daclizumab)	CD25	Aug-2003	Increased mortality	N
			Hypersensitivity reactions	N

Finally, there are two fully human antibodies (identified by the substem -u) with reported Medwatch safety alerts. They are presented in Table 6, below (adalimumab and golimumab – while adalimumab being presented by alerts reported for itself individually as well as for its therapeutic group of TNF-α blockers).

Table 6: Fully human antibody alerts

Name	Target	Alert Date	Alert Message	Observed (Yes/No)
Humira (adalimumab)	TNF-α	Nov 2004	Infections with the combined use of anakinra	N
			Hypersensitivity reactions, including anaphylaxis	N
			Hematologic events, including pancytopenia and aplastic anemia	Y
TNF-α blockers (Humira and Simponi (golimumab))	TNF-α	Jun-2008	Lymphoma and other cancers in children and young adults	Humira: Y
		Sep-2008	Histoplasmosis, coccidioidomycosis, blastomycosis and other opportunistic infections	Humira: Y
		May-2009	Histoplasmosis and other invasive fungal infections	Humira: Y
		Aug-2009	Lymphoma and other cancers in children and young adults	Humira: Y Simponi: Y

6.2 Time to safety alerts

Time to publication of safety alerts was assessed by means of Kaplan-Meier estimate, using MedCalc software. The time coverage was extended and Medwatch safety alerts for therapeutic monoclonal antibodies up to Sep-2013, inclusive (up to the 3rd quarter of 2013) have been included in the analysis. Note that the observation period in the analysis of time to reaction has been extended with respect to the analysis of predictability performed in the previous step.

Hazard is a measure of how rapidly the event of interest occurs. The hazard ratio compares the hazards in two groups. Hazard ratio (95% CI) demonstrated by the Kaplan-Meier analysis is 1.0076 (0.6388 to 1.5893) for observed vs 0.9925 (0.6292 to 1.5655) for the unobserved alerts, as demonstrated in Table 7. Note that the computation of the hazard ratio assumes that the ratio is consistent over time. Therefore, if the survival curves cross, the hazard ratio statistic should be ignored. In the current example, the curves cross on several occasions, and the overall hazard ratio does not provide a useful comparison between the curves.

Table 7: Kaplan-Meier Hazard ratios[a] with 95% Confidence Interval

Factor	Unobserved	Observed
Unobserved	-	1.0076 0.6388 to 1.5893
Observed	0.9925 0.6292 to 1.5655	-

[a] Column/Row

The statistics of time to safety alert for individual drug obtained by the Kaplan-Meier analysis are presented in Table 8.

Table 8: Kaplan-Meier survival curve statistics

	Factor				Overall	
	Unobserved		Observed			
Survival time	Survival Proportion	Standard Error	Survival Proportion	Standard Error	Survival Proportion	Standard Error
1	-	-	0.976	0.0235	0.987	0.0132
2	0.970	0.0298	0.952	0.0329	0.960	0.0226
3	0.939	0.0415	-	-	0.947	0.0259
4	-	-	0.929	0.0397	0.933	0.0288
5	0.909	0.0500	0.881	0.0500	0.893	0.0356
6	-	-	0.857	0.0540	0.880	0.0375
8	-	-	0.833	0.0575	0.867	0.0393
11	-	-	0.810	0.0606	0.853	0.0409
12	-	-	0.786	0.0633	0.840	0.0423
13	-	-	0.762	0.0657	0.827	0.0437
16	0.818	0.0671	0.738	0.0678	0.773	0.0483
17	0.788	0.0712	0.714	0.0697	0.747	0.0502
18	0.758	0.0746	-	-	0.733	0.0511
21	0.697	0.0800	0.619	0.0749	0.653	0.0550
24	-	-	0.595	0.0757	0.640	0.0554
29	0.667	0.0821	0.571	0.0764	0.613	0.0562
31	0.606	0.0851	-	-	0.587	0.0569
36	0.576	0.0860	-	-	0.573	0.0571
38	0.515	0.0870	0.548	0.0768	0.533	0.0576
39	-	-	0.524	0.0771	0.520	0.0577
41	-	-	0.500	0.0772	0.507	0.0577
44	-	-	0.452	0.0768	0.480	0.0577
45	0.485	0.0870	-	-	0.467	0.0576
47	-	-	0.429	0.0764	0.453	0.0575
53	0.455	0.0867	0.405	0.0757	0.427	0.0571
58	0.424	0.0860	-	-	0.413	0.0569
60	-	-	0.381	0.0749	0.400	0.0566
64	-	-	0.357	0.0739	0.387	0.0562
67	-	-	0.333	0.0727	0.373	0.0559
68	0.364	0.0837	-	-	0.347	0.0550
69	-	-	0.310	0.0713	0.333	0.0544
72	-	-	0.286	0.0697	0.320	0.0539
73	0.182	0.0671	-	-	0.240	0.0493
76	-	-	0.262	0.0678	0.227	0.0483
77	0.152	0.0624	0.238	0.0657	0.200	0.0462
80	-	-	0.214	0.0633	0.187	0.0450
83	0.121	0.0568	-	-	0.173	0.0437
84	-	-	0.190	0.0606	0.160	0.0423
86	0.0909	0.0500	-	-	0.147	0.0409
100	-	-	0.167	0.0575	0.133	0.0393
105	-	-	0.143	0.0540	0.120	0.0375
109	0.0606	0.0415	-	-	0.107	0.0356
119	-	-	0.119	0.0500	0.0933	0.0336
121	-	-	0.0952	0.0453	0.0800	0.0313
129	-	-	0.0714	0.0397	0.0667	0.0288
130	0.0303	0.0298	-	-	0.0533	0.0259
132	-	-	0.0476	0.0329	0.0400	0.0226
152	-	-	0.0238	0.0235	0.0267	0.0186
157	-	-	0.000	0.000	0.0133	0.0132
180	0.000	0.000	-	-	0.000	0.000

Endpoint: Observed n	33.0	42.0	
Expected n	33.1	41.9	
Observed/Expected	0.9958	1.0033	

Up to 31-Sep-2013 there were altogether 75 alerts: 42 assessed as observed vs 33 unobserved. Median time to alert (median survival) is somewhat shorter for observed alerts (labelled Y) with respect to unobserved (labelled N): with 41 and 45 months, respectively The mean time to survival is almost the same: with 52.548 and 52.515 months, respectively (see The Chi-square statistic gives a value of 0.001131. The statistical difference using logrank test is non-significant with a P = 0.9732 (see Table 10).

Table 9)

The Chi-square statistic gives a value of 0.001131. The statistical difference using logrank test is non-significant with a P = 0.9732 (see Table 10).

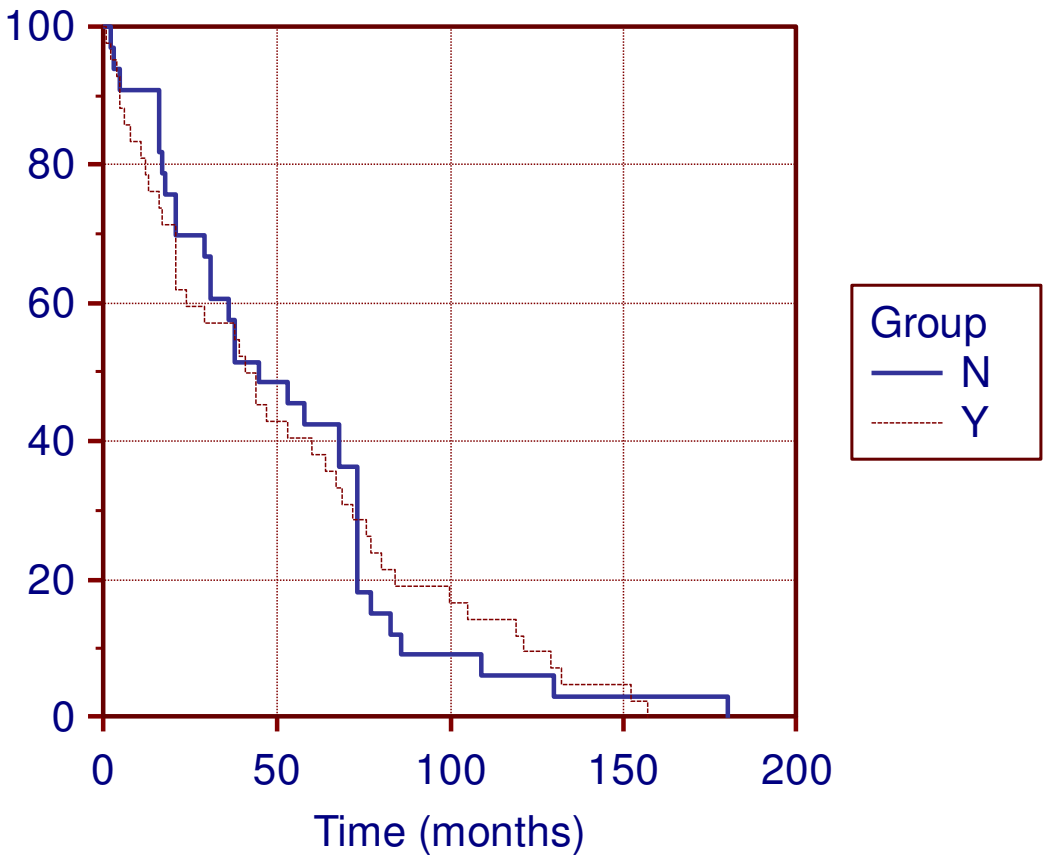
Table 9: Kaplan-Meier mean and median survival and statistic (Logrank test)

Factor	Mean	SE	95% CI for the mean	Median	95% CI for the median
Unobserved	52.515	6.712	39.360 to 65.670	45.000	31.000 to 73.000
Observed	52.548	6.769	39.280 to 65.815	41.000	21.000 to 67.000
Overall	52.533	3.271	46.122 to 58.945	44.000	31.000 to 64.000

Chi-squared	0.001131
DF	1
Significance	P = 0.9732

The absence of statistical difference is demonstrated by the graphical representation of the curves which are almost super-imposable (see Figure 6).

Figure 6: Kaplan-Meier survival curve of time to Medwatch safety alert



Legend: Observed alerts (labelled Y); Unobserved alerts (labelled N);

As demonstrated, the difference between the observed and unobserved alerts is statistically not significant. However, even if not demonstrable by overall statistics over the entire period covered, the graph shows that there is a slight tendency of earlier reporting of observed alerts in the initial phases after marketing authorisation. The percentage of alerts in the group of observable alerts is consistently higher in the early phases with respect to unobserved alerts (there are "survivors" in the groups N). This applies to about three quarters of the total number of alerts in this analysis. On the contrary, this difference does not persist starting at about 72 months i.e. 6 years into the observation. After this point there is tendency of more unobserved alerts in the later phases. Even in the absence of statistical significance of this finding, the clinical or public health significance may be relevant.

Table 10: Time to safety alert

Name	Alert Date	Alert Message	Observed (Yes/No)	Approval date	Months to alert
Adcetris (brentuximab vedotin)	Jan-12	PML	**N**	Aug-2011	5
		Pulmonary toxicity (interaction)	**Y**		5
Arzerra (ofatumumab)	Sep-2013	Recommendations to Decrease Risk of Hepatitis B Reactivation	**Y**	Oct-2009	47
Avastin (bevacizumab)	Aug-2004	Arterial thromboembolic events	**Y**	Feb-2004	6
	Jan-2005	Arterial thromboembolic events.	**Y**		11
	Sep-2006	Reversible posterior leukoencephalopathy syndrome	**N**		31
		Nasal septum perforation.	**N**		31
	April-2007	Tracheoesophageal fistula	**N**		38
	Jul-2008	Microangiopathic hemolytic anemia in combination with sunitinib malate	**N**		53
Erbitux (cetuximab)	Sep-2005	Observation periods following infusion	**Y**	Feb-2004	17
		Hypomagnesemia	**N**		17
Herceptin (trastuzumab)	May-2000	Hypersensitivity reactions	**Y**	Aug-1998	21
		Infusion reactions	**Y**		21
		Pulmonary reactions	**N**		21
	Aug-2005	Cardiotoxicity	**Y**		84

Humira (adalimumab)	Nov-2004	Infections with the combined use of anakinra	**N**	Dec 2002	16
		Hypersensitivity reactions, including anaphylaxis	**N**		16
		Hematologic events, including pancytopenia and aplastic anemia	**Y**		16
Lucentis (ranibizumab)	Feb-2007	Stroke higher incidence with higher dose	**Y**	Jun-2006	8
NeutroSpec (Technetium fanolesomab)	Dec-2005	Cardiopulmonary events (market suspension)	**N**	Jun-2004	18
Raptiva (efalizumab)	Jul-2005	Immune-mediated hemolytic anemia	**N**	Oct-2003	21
		Thrombocytopenia	**Y**		21
		Infections	**Y**		21
	Oct-2008	PML, fungal and other opportunistic infections	**Y**		60
	Feb-2009	PML (market suspension)	**Y**		64
Remicade (infliximab)	Oct-1998	Adverse events due to antibodies	**Y**	Aug-1998	2
	Oct-2001	Tuberculosis and other opportunistic infections	**Y**		38
		Heart failure	**N**		38
	Aug-2004	Lymphoma and other malignancies	**Y**		72
	Dec-2004	Hepatic reactions	**Y**		76
Rituxan (rituximab)	Nov-1998	Infusion reactions	**Y**	Nov-1997	12

	Oct-2004	Hepatitis B virus reactivation	**N**		83
	Dec-2006	PML	**N**		109
	Sep-2008	PML leading to death18 months after the last dose	**N**		130
	Sep-2013	Recommendations to Decrease Risk of Hepatitis B Reactivation	**N**		180
Simulect (basiliximab)	Oct-2000	Hypersensitivity reactions, including anaphylaxis	**N**	May-1998	29
Synagis (palivizumab)	Nov 2002	Anaphylaxis	**Y**	Jun-1998	53
Cimzia:	Jun-2008	TNF-α Blockers (Remicade, Humira, Cimzia (certolizumab pegol)) Lymphoma and other cancers in children and young adults	**N**	Apr-2008	2
Humira:			**Y**	Dec 2002	67
Remicade:			**Y**	Aug-1998	119
Cimzia:	Sep-2008	TNF-α Blockers (Remicade, Humira, Cimzia) Histoplasmosis, coccidioidomycosis, blastomycosis and other opportunistic infections	**Y**	Apr-2008	2
Humira:			**Y**	Dec 2002	66
Remicade:			**Y**	Aug-1998	118
Cimzia:	May-2009	TNF-α Blockers (Remicade, Humira, Cimzia) Histoplasmosis and other invasive fungal infections	**Y**	Apr-2008	13
Humira:			**Y**	Dec 2002	77
Remicade:			**Y**	Aug-1998	129
Cimzia:	Aug-2009	TNF-α Blockers (Remicade, Humira, Cimzia, and Simponi (golimumab)) Lymphoma and other cancers in children and young adults	**N**	Apr-2008	16
Humira:			**Y**	Dec 2002	80
Remicade:			**Y**	Aug-1998	132
Simponi:			**Y**	Apr-2009	4

Cimzia:	Sep-2011	TNF-α Blockers (Remicade, Humira, Cimzia, and Simponi (golimumab)) Risk of Infection from Legionella and Listeria	**N**	Apr-2008	41
Humira:			**Y**	Dec 2002	105
Remicade:			**Y**	Aug-1998	157
Simponi:			**Y**	Apr-2009	29
Cimzia:	Apr-2011	TNF-α Blockers (Remicade, Humira, Cimzia, and Simponi (golimumab)) Hepatosplenic T-Cell Lymphoma	**N**	Apr-2008	36
Humira:			**Y**	Dec 2002	152
Remicade:			**Y**	Aug-1998	100
Simponi:			**Y**	Apr-2009	24
Tysabri (natalizumab)	Feb-2005	PML	**N**	Nov-2004	3
	Feb-2008	Liver injury	**Y**		39
	Aug-2008	PML	**N**		45
	Sep-2009	PML	**N**		58
	Apr-2011	PML	**N**		77
	Jan-2012	PML	**N**		86
Xolair (omalizumab)	Feb-2007 Jul-2009	Anaphylaxis	**Y**	Jun-2003	44
		Ischemic heart disease	**N**		73
		Arrhythmias	**N**		73
		Cardiomyopathy and cardiac failure	**N**		73
		Pulmonary hypertension	**N**		73
		Cerebrovascular disorders	**N**		73

		Embolic, thrombotic and thrombophlebitic events	**N**		73
Yervoy (ipilimumab)	Mar-2011	Severe Immune-Mediated Adverse Reactions	**Y**	Jun-2011	1
Zenapax (daclizumab)	Aug-2003	Increased mortality	**N**	Dec-1997	68
		Hypersensitivity reactions	**N**		68
Zevalin (ibritumomab tiuxetan)	Oct-2005	Cutaneous or mucocutaneous reactions	**Y**	Feb-2002	44

7 DISCUSSION

7.1 Predictability of serious adverse reaction alerts for monoclonal antibodies

7.1.1 Source and reliability of data

The percentage of observed events in our sample of Medwatch safety alerts should be interpreted in the light of the bias of incomplete and selective "spontaneous" reporting of suspected reactions. A reaction not previously identified is more likely to be considered coincidental, the medication not implicated as causative and adverse event not worthy of spontaneous reporting. It may follow that there are more unpredictable ADRs which are more difficult to detect which have so far not been detected. However, a lot of alerts are also based on post-approval studies where reporting is mandatory, thus partially eliminating the bias.

The absence of safety alerts to certain mAbs may be an indicator of their safety, but also of on-going review due to recent marketing, or limited use. Hence, it does not mean that the mAbs for which no alerts are issued are safe or comparatively safer than the ones or which alerts are issued.

Many ADRs are detected only after decades or centuries of use. One can take acetylsalicylic acid as an example. This medication has been used since antiquity. The Ebers papyrus, which has been dated to circa 1500 BC, verifies that the ancient Egyptians were also aware of the antipyretic property of willow leaves and used them to treat various inflammatory disorders. Aspirin (acetylsalicylic acid) was synthesized based on the active principles isolated from the willow tree. It is one of the first modern medicines which has been used since the late 19th century. Nevertheless, a common ADR such as the Reye's syndrome (hepatoencephalopathy following aspirin use in febrile illnesses in children) has been reported only in 1963 (88).

Another more recent striking example of failure to identify a common ADR before marketing is dry cough with the use of angiotensin converting enzyme (ACE) inhibitors. Cough is now recognized as an ADR occurring in up to 15% of treated patients (89), yet such a frequent adverse event was only recognized 4 years after captopril marketing authorization (90). One can only imagine the discomfort to patients, healthcare expense and health hazards due to diagnostic and therapeutic measures before this minor but frequent ADR was recognized.

The two examples presented above prove two arguments: the case of Aspirin proves that common yet unpredictable (and as yet unexplainable) ADRs can go undetected for very long

years. The example of ACE inhibitor-induced cough shows that even very predictable and very common ADRs can escape detection even to modern medicine. At the time of marketing of ACE inhibitors, pharmacovigilance was already a fairly well established discipline and medical profession well alert of side-effects.

Both arguments highlight the number of yet undetected ADRs. This figure of undetected ADRs is presumably expected to be more significant for the unpredictable reactions; hence the true number and percentage of the unobserved reactions is likely to be higher than demonstrated by the results presented here. However, the proof of this assumption is likely to eternally remain in the realm of the unknown.

7.1.2 Pattern of adverse reactions

Non-specific toxicity such as hypersensitivity was reported as alert for 6 mAbs; half of them observed pre-approval. Hypersensitivity may have been anticipated as all 6 mAbs elicited immune response leading to development of anti-drug antibodies in pre-approval trials.

Clustering of vascular thromboembolic adverse reaction types were observed in the case of bevacizumab and ranibizumab, but also in other non-mAb drugs targeting VEGF (86). Once a relevant pathological process is identified, multiple mAbs (92) and other drugs targeting it are likely to be developed; hence it is likely that a similar "target-related toxicity" (93) clustering will continue to be observed.

According to traditional classification applied to conventional drugs, ad verse reactions due to exaggerated pharmacological action were described as type A (augmented) adverse reactions, (94) whereas target-related toxicity is an appropriate equivalent term. A unique characteristic of mAbs, however, is their capacity to exert actions through their Fc region following target binding. These actions include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and antibody-dependent cellular phagocytosis. Recruitment of these effectors is de pendent on the isotype of the antibody, and its ability to recruit complement or effector cells (96). Toxicity evaluation should, therefore, take into account not only target distribution and function on various tissues, as in conventional drugs, but also additional Fc region activity. Consequently, ADRs predictability can be further extended to cover Fc region. This was not performed as part of this thesis. Even though this could and should be part of predictability, since this would introduce a high level subjectivity to the assessment of predictability due to the an extended imprecisely defined range of ADRs which could be

assessed as predictable based on this mechanism. However, this evaluation by MAH, prescribers as well as regulatory agencies is required for all individual mAbs.

Non-specific toxicity such as hypersensitivity is very much equivalent to type B (bizarre) reactions described for conventional drugs. A high number of alerts for infections and malignancies should not be interpreted as an inherent propensity of mAbs to de crease immunity, but rather a property of currently marketed mAbs targeting the immune system. Indeed, for 5 out of 7 mAbs with infection alerts, the increased rate of infection was identified pre-approval, the exceptions being rituximab and natalizumab. Alerts for malignancies were issued for infliximab and adalimumab (observed) and certolizumab pegol (not observed).

Breakdown based on the origin of the antibody i.e. the species from which the antibody is obtained did not show a clear difference in the pattern of alerts reported for various mAb subcategories. This is most likely due to the small sample size in each of the subcategories. It is well established that biotech products containing more foreign (non-human) amino acid sequences are more immunogenic. However, the two murine antibodies presenting alerts in this sample (technetium fanolesomab and ibritumomab tiuxetan) are at the same time linked to radioactive isotopes, which affect the overall safety profile of the product.

7.2 Kaplan-Meier "survival analysis"

The analysis demonstrated that observed alerts are reported earlier. This indicates the potential value of risk management planning in the identification of safety concerns for which a suspicion has been previously raised. On the contrary, safety concerns for which suspicion has not been raised prior to marketing require more time of observation before they are detected.

The analysis was performed with a number of modifications to its original purpose, as described in the section 5 Methods. Its use has been extended outside of its common application to an unconventional type of data. Sample data is not a typical clinical trial or epidemiological study. A detail is often overlooked in medical statistics is that the original use of the method is not restricted to what the method is commonly used for nowadays. Edward L. Kaplan actually proposed the method while working on the lifetimes of vacuum tubes in the repeaters in telephone cables buried in the ocean, as part of Bell Telephone Laboratories (95). Despite the significant list of modifications to the common use, the Kaplan-Meier

survival analysis nevertheless provides useful analysis, as it offers an insight into the dynamics of detection of safety alerts.

The two curves in the analysis show non-significant difference, but this finding should be interpreted taking into account the type of data analysed. All subject i.e. alerts in this analysis reach an endpoint. In fact, alerts are only assigned one of the two groups (observed vs unobserved) once the endpoint has been reached. Hence it is understandable that there should be no statistical difference overall. What the analysis is useful for is the timing to the detection of alerts. For observed alerts, both the mean and median time show a trend towards early reporting of observed alerts. The standard deviations are also very large, given the type of data observed, and demonstrating statistical at this level is difficult. The detection of even the trends is valuable in this context.

Analysis of time to detection of an important safety finding is of utmost importance to public health. Extension of the Kaplan-Meier analysis to cover the type of data it has not been designed for will require additional validation. But even in the absence of statistical or regulatory validation, it is an illustrative way of presenting the time to safety alerts. The analysis does not prove that predictable reactions can be observed any earlier, but indicates certain value in its evaluation.

7.3 Limitations

7.3.1 Limitations of predictability evaluation

A limitation of this predictability evaluation may be the lack of cross comparison with alerts for small molecules and other biopharmaceutical in the same time interval. However, Giezen et al. recently demonstrated that the first biopharmaceuticals approved in a chemical, pharmacological, and therapeutic subgroup were at a higher risk for their first safety-related regulatory action (97). As mAbs are a new subgroup, such a comparative safety study would have to account for not only the evolving safety profile, but also regulatory authority practices for issuing safety alerts for a new subgroup. Monoclonal antibodies are also generally more likely to be developed to treat serious illnesses, and serious illnesses themselves are fertile ground for “toxicity” whether related to the drug or disease, so interpreting data on the drug’s or biopharmaceuticals’s risk must take that into account (98). In addition, this review focussed on Medwatch alerts which are generally issued for serious reactions, so that mild or but frequent reactions may have been omitted.

Limitation of the objectivity of the assessment of alerts as observed vs unobserved should also be considered. Several examples of difficulties in assessment were presented in the Results section. It should be noted that this approach to assessing predictability has to the best of the author's knowledge not been previously attempted in published literature. The reason such an approach has not been attempted maybe due to the difficulties in the assessment and individualized approach to each alert. Information on drug labelling and Medwatch safety alerts were taken as the basis of analysis of the evolving safety profile. Such documents are products of a comprehensive overview of product safety information vigilantly scrutinized by both the manufacturer and the FDA. The author believes that subjectivity has been successfully eliminated by strict application of assessment criteria.

A more subjective supplementary analysis was undertaken to classify certain alerts as unobserved but arguably predictable based mechanism of action or antibody target. Such analysis is regarded as a sensitivity analysis and is subject to interpretation of the extent that an adverse reaction may be predictable based on mechanism of action. Objectivisation of such analysis can be made based on predifened criteria of suspicion of reaction. An ideally pre-defined suspicion would be a risk management plans specifying potential risks. In the absence of complete information, such an analysis remains informative, but partial and incompletely objective.

7.3.2 Limitations of Kaplan-Meier estimate

In the case of data presented here, the "survival" is considered the time to regulatory alert i.e. the time to the important safety finding of any kind which is significant enough to justify an alert to health professionals. Another modification of the Kaplan-Meier analysis is that there is no loss of subjects i.e. there are no drop-outs from the study and there is no loss to follow-up. Even though Kaplan-Meier analysis can account for loss of subjects, the analysis remains valid even in case of no loss. And the final modification of the analysis is that subjects (medications i.e. alerts) are assigned to one of the two groups (predictable vs unpredictable alerts) at the time the event occurred, and not based on initial allocation to one of the two groups. This way, all the subjects experience an event. Furthermore, one same subject (medication) may experience more than one event which classifies it in both groups at the same time.

7.4 Predictive methods

There is a multitude of available predictive methods. Many of these methods showed value, but none of them alone are sufficiently predictive. Clinical use of biopharmaceutical will need to continue relying on multiple methods. Immunological assays provide evidence of sensitization to a specific drug but must always be interpreted within the appropriate clinical context.

It is important to emphasize here that the immunological assays are only a part of the overall immunogenicity assessment. A positive ADA result means that antibody was detected and a negative result means that antibody was not detected under the conditions of the analysis. The detection of ADA, or lack thereof, should be considered with other study parameters such as pharmacokinetics, pharmacodynamics, and adverse event data to determine if immunogenicity is of concern to the patient population. If the ADA result is "negative" in parallel with declining PK and/or pharmacodynamic (PD) values, the ADA may have been undetectable due to sensitivity, specificity, or interference factors not controlled for in the assay. Additional sample treatment may be necessary to clarify ADA status. Alternatively, if the ADA result is "positive" in parallel with unchanged PK/PD profile and absence of related adverse events, the safety assessment of the presence of ADA will again rely upon the risk-based approach. Obviously, these considerations apply when appropriate PK/PD data are available.

Anti-drug antibodies form the corner stone of immunogenicity assessment. They are mandatory according to EMA guidelines (58) and recommended by scientific boards elsewhere (15, 60). The advantages of antibody assessment are numerous, summarizing the key elements:

- The tests are reproducible based on a standardized assay.
- The test carries no risk to the patient, excluding the minimal risk, minimally invasive blood sampling.
- Observer subjectivity does not play a role. Results obtained provided a numeric value of antibody titres hence eliminating elements of subjectivity.
- Evaluation of results does not require repeated or continuous presence of the patient

(as a contrast to skin testing where the patient needs to be re-examined after a given period of time after the administration). Hence the test is convenient for the patient.

- Concomitant drugs given at the time of testing do not have an influence on the test results. It should be noted that concomitant medication, such as concomitant immunosuppressant, given at the time of drug administration may have an effect on antibody formation.
- In addition to confirmation of the presence of ADAs, their further characterisation and antibody typing can be performed. The antibody typing may suggest the type of reactions which are possible based on the antibody profile obtained.

Despite the advantages of antibody testing, evaluation of other test should be considered according to current regulatory requirements in the European Union (58). Antibody testing is considered a regulatory standard, but should be no means be viewed as the only method. Antibody testing can miss other types of immune reactions such as cell-mediated immunity. Skin testing was discussed in some detail. Skin tests appear to be equally useful for biopharmaceuticals as for small molecule drugs, but they are used to a very limited degree. The low number of published articles on skin testing with biopharmaceuticals is surprising. In stark contrast, the data on small molecules are abundant, and leads one to question the reasons. It is possible that there is some adherence to established methods. The availability of standardized formulations of allergens certainly contributes to continued use of skin testing for traditional indications.

Highlighting the advantages of skin tests:

- Skin tests are relatively easy to perform;
- They do not require any specialized equipment, reagents, standardized biochemical assays.
- As a result of the elements presented above, it follows that the cost of performing skin tests is low.
- Some quantification of the size of local injection site reaction is possible, but quantification of the extent of urticaria or reported clinical symptoms allows room for reader interpretation. This feature, however, is a disadvantage and advantage at the same time as it provides an observable and evaluable reaction to drug administration

under controlled testing conditions.

Highlighting the disadvantages of skin tests:

- Wash-out period for certain concomitant drugs is required, which may not always be possible if stable regular treatment with the concomitant drug is required.
- There is a risk of possible further stimulation of the immune response
- Formulation and concentrations of the drug is relevant in order to differentiate between purely irritant and immune reactions.
- The time of observations can vary depending on the type of reaction. Immediate reactions tend to appear within hours, whereas delayed reaction may be observed only several days later. This feature, again, is a disadvantage and advantage at the same time since this allows the identification of delayed hypersensitivity immune reactions.

Skin tests were the method of choice at the time before the more complex approach of detection of ADA for biopharmaceuticals was put in place as a regulatory standard. In parallel, the abundance of published data on small-molecule allergy testing appears to suggest that small-molecule allergy primarily relies on skin rather than ADA testing.

It is questionable if this preference of one testing of another for small-molecule vs biopharmaceuticals is based on science or tradition and adherence to commonly used methods. Despite all of the above mentioned limitations, it still appears that skin testing is underused for biopharmaceuticals. Both pre-approval and post-approval testing in parallel to ADA testing may prove their value.

Other in vitro tests are available, such as BAT or LTT. Their use depends on the specific type of reaction and they should be used in specific situations, as their applicability should be validated for each drug.

In addition to imposing additional requirements for drug developers and marketing authorization holders, a RMP must take into account the reasonable burden and the rewards for establishing a risk management system which includes additional tests. An antibody assay developed for a drug may be regarded as a separate product and the drug MAH may in parallel seek marketing authorization for the assay. The assays are invariably available as they

are mandated as part of pre-authorisation testing. These assays were developed for search purposes, and may not always be suitable for routine laboratory application. However, in some cases it may be possible to make minimal adjustment to the test to make them marketable. Alliteratively, in case of cumbersome tests, or cases where a specific equipment or cell lines are required, regional centres of excellence may perform the testing.The frequency and the extent of testing should be dictated by the risk of adverse reaction. Such a "risk-based" approach has been recommended by Shankar et al (60) and should form the foundation of the testing. For low risk products, neither the expense, nor inconvenience for patients justifies frequent and routine testing. In low risk cases the testing should be restricted to patients in which an adverse reaction is suspected or has been documented, yet the benefit of the drug is still be believed to outweigh the risk. Therefore, not the whole population; but a selected number of patient are at risk justifying testing in accordance with the risk-based strategy.

The principle of prediction equally applies to both conventional drugs and biopharmaceuticals. The difference is essentially the methods applied. For conventional drugs metabolism and metabolic interactions would more commonly play a role, while immunogenicity is not routinely an issue.

A pharmacoeconomic analysis is outside of the scope of this article. Pharmacoeconomic analysis is quite specific to healthcare systems and would not be applicable globally. Nevertheless, it is likely that the cost of treating ADRs and the cost of the drug itself would in many cases outweigh the cost of immunogenicity testing. The drug MAH could be motivated to provide a test as a companion to the drug. If an antibody essay is developed and validated for clinical trial purposes, and anyway available, adaptation of the assay for commercial purposes may be possible. This effort would enable identification of patients at risk and patients with a lower likelihood of reactions. Ultimately, all involved parties could benefit: the MAH, health insurance and last but absolutely not the least, the patients.

7.5 Rechallenge

The most appropriate way to determine the predictive value of any test would be to compare it to the results of a controlled rechallenge. For ethical reasons the positive predictive value of

for many drugs cannot be precisely evaluated as challenge testing may provoke life-threatening reactions.

When considering intentional rechallenge, one should take into account the benefit-risk balance of the suspected causative medication, and the benefit-risk balance of the best available alternative treatment or no treatment. Rechallenge is unacceptable for purely scientific aims and acceptable only when it could be beneficial to the individual subject under evaluation. Council of International Organization of Medical Sciences (CIOMS), a global think tank on drug safety, considers rechallenge unacceptable for purely scientific aims and acceptable only when it could be beneficial to an individual subject (99). The US FDA: Guidance for Industry on Drug-Induced Liver Injury (100) provides examples of hepatic adverse reactions which are more prone to recur with reexposure e.g. cases showing indicators of immunological reaction such as eosinophilia, rash, fever, or other symptoms or findings. Many well-considered recommendations in this guidance could extend to intentional rechallenge with reactions other than liver injury. Most notably, the requirement of assessment of gravity of the initial reaction, close observation of the patient on rechallenge and patient informed consent.

Justifying rechallenge depends on examining evidence to see whether there is no available alternative which may confer the same benefit. A prescriber should further examine the risk in detail and the potential predictive risk factors for an individual reaction so that risk minimisation measures can be implemented, where possible. These risk minimization measures are not only specific to each drug, but even more, they should be specifically tailored to each significant adverse reaction and specifically interpreted for each individual patient (101). In routine clinical practice this would apply to adverse reactions which most frequently lead to treatment discontinuation.

A particular form of rechallenge is desensitisation i.e. induction of temporary clinical unresponsiveness to drug antigens which caused severe hypersensitivity reactions. In this case, desensitisation procedure itself is intentional rechallenge.

The need to consider rechallenge after a suspected reaction applies through clinical drug development to marketing. Sponsors could create rechallenge algorithms in the pre-authorisation phases as part of a developmental RMP. In early phases of development both risks and benefits may be poorly characterized, further complicating rechallenge

considerations. Because of liability concerns, licence holders would be understandably reluctant to create rechallenge algorithms, unless they were part of an approved RMP.

Prescribing physicians should not act in isolation. They should carefully document their actions systematically in risky situations, such as intentional rechallenge. At the present time, intentional and recurrent rechallenge in a patient series is often performed by specialist centres which have available personnel for monitoring and intensive care e.g. allergy centres performing desensitisation. For marketed drugs, expert societies or specialist centres would be most competent to further develop algorithms to support prescribing professionals. In their absence, patients may continue to be exposed to random benefit/risk assessment by physicians who may not be fully informed of evidence-based recommendations. Whatever the context may be, the best practice for rechallenge should be considered and guidance provided to individual prescribers.

Before attempting rechallenge the treating physician must consider the following: Treatment benefit, treatment risk, risk mitigation and ethical aspects (patient information and consent) (See Figure 7):

Benefit assessment:

- The real need of the drug: Often the adverse reaction happens with a drug that has valid alternatives. The administration of the alternative drug may be without discomfort and should not have a higher probability to induce ADR. In presence of a valid alternative, rechallenge is not ethically justified.

- Benefit assessment for both suspect and alternative treatment should be based on clinical trial efficacy results in the given patient population. The effectiveness for the particular patient might also be known if the patient was treated long enough to assess it. The alternative treatment is assessed similarly as the suspect drug, the key difference being that there may not have been a previous challenge with the alternative treatment and the patient's response to alternative treatment may be unknown. If both suspect and alternative drugs are from the same class or cause similar adverse reactions (for example bleeding events in deciding between two anticoagulants), a comparative estimate of both benefit and risk may be possible. CIOMS Working

Group IV provides good general guidance on benefit and risk assessment (103).

- The acceptable level of risk should be justified by the expected benefit. The final result of a benefit assessment is a defined threshold above which the risk is not justified by the expected benefit. Complete withdrawal of medical treatment may be considered. In such case, the risk of disease worsening and progression in the absence of treatment should be taken into account instead of the risk of alternative treatment.

Risk assessment:

- Estimate the characteristics of the reaction which may occur upon rechallenge: This is particularly the case for immunological hypersensitivity reactions in which prior sensitisation has occurred, as the reaction may occur earlier and may be more severe on rechallenge. The mechanism of adverse reaction may or may not be known, the severity, time of onset, response to previous treatment of the reaction should be considered.

- Note that the risk of rechallenge is not equivalent to the risk of the initial reaction which caused the suspicion that the drug was causative. Instead, the risk of rechallenge is the reasonably expected risk of the reaction if it were to reoccur and that it might be more severe should it occur again. Likelihood that the suspect drug was causative is essential at this point.

- Identify patients with risk factors based on characteristics of the initial reaction and medical history which may predispose to higher risk: Pharmacogenomic studies, in particular, have been able to identify strong genetic predisposing factors for hypersensitivity reactions to carbamazepine, abacavir and allopurinol. With the expanding use of pharmacogenomics, this list will surely continue to expand (104). For biopharmaceutical, the range of predictive factors discussed above should be considered including, anti-drug antibodies, skin tests, etc.

Risk mitigation:

- Identify appropriate prophylaxis based on best available evidence to mitigate or prevent the anticipated reaction, even though this may be limited to ‘expert opinion’. The best treatment of the reaction may not be known, but should be proactively considered.

- Define the requirements for monitoring a possible adverse reaction such as the variables to be measured, the interval of testing and examination and a point in time at which further monitoring may no longer be required. Ensuring follow-up at appropriate intervals is particularly applicable to reactions which take longer time to develop and for which certain laboratory or clinical markers can be used for early identification. Such a monitoring schedule is likely to be more intensive than what is currently in the manufacturers' product information leaflets. Hence, a structured and adapted written plan of action based on the algorithm is recommended. Such a plan would serve a dual purpose: as a basis for explaining an individual physician's action to a local ethics committee and as a practical guide for the clinician and their team which can be recorded in the patient's notes.

- Performing rechallenge under controlled conditions (e.g. inpatient hospitalisation during rechallenge, intensive care unit) minimises the risk if the precautions are taken to prevent or promptly treat the recurring reaction. Qualified and informed personnel and appropriate diagnostic and potentially required therapeutic measures should be readily available. This particularly applies to immediate reactions e.g. immediate hypersensitivity reactions. This approach must be rational, depending on the expected time of insurgence of the reaction: late reactions need prolonged observations. Reinitiating administration at a lower dose and gradually increasing it may help minimise the risk. Usually the administration should reach the therapeutic dosage, to exclude a possible reaction at higher doses.

Information and consent:

- We emphasise the importance of patients (and their families in case of patient mental incapacity) to provide informed consent to rechallenge (once the rationale of the need of rechallenge is defined). The consent should provide information on the benefit/risk balance of the available treatment alternatives.

- The informed consent and the rechallenge algorithm may require approval from an appropriate clinical ethics committee or other comparable body monitoring prescribing decisions. This depends on the specific case, local requirements as well as the settings e.g. routine clinical use or clinical trials. In any case, rechallenge cannot be performed without due ethical consideration for patient autonomy so that acts of rechallenge must

be intentional, voluntary and based on full understanding of the circumstances.

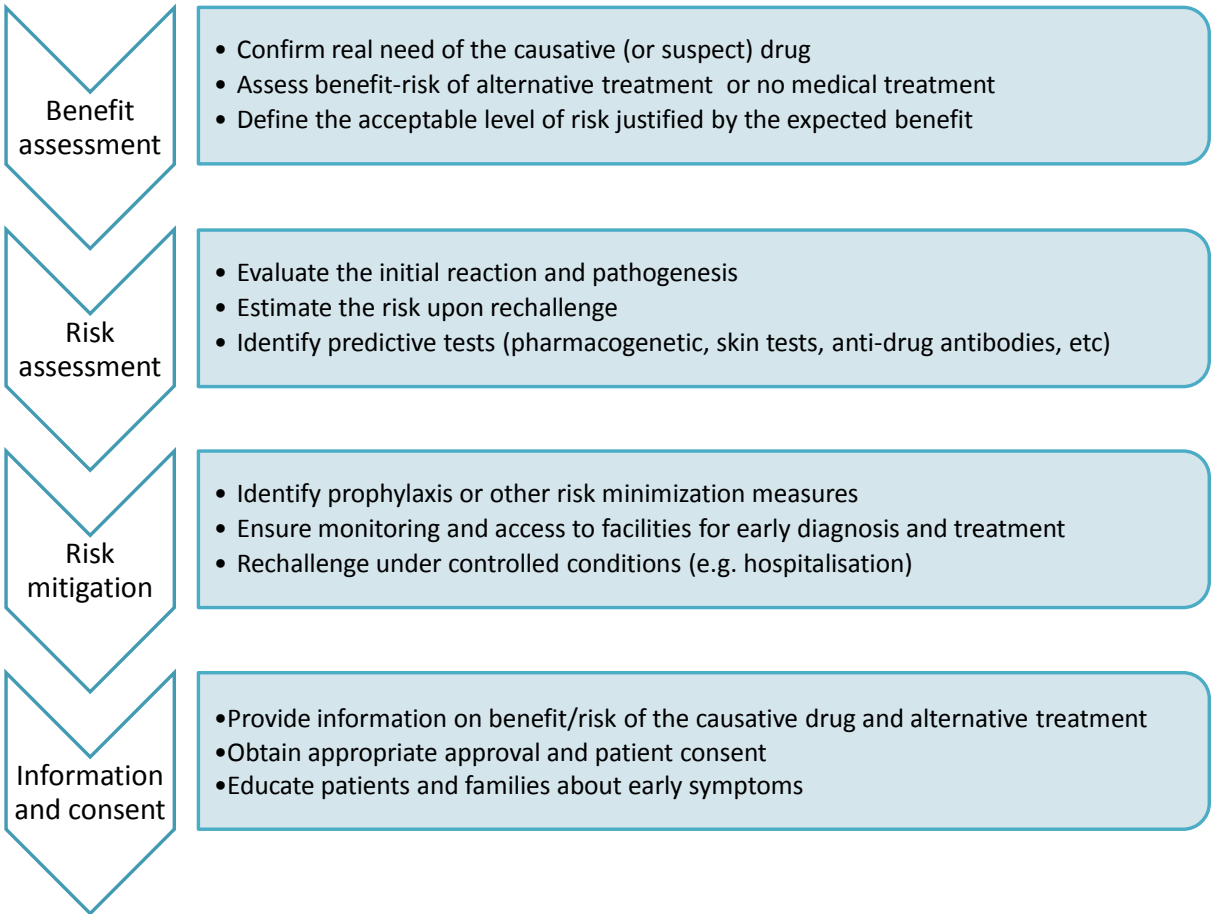
- Authorised patient information leaflet should already contain general information on expected adverse reactions. However, this information may need to be supplemented by additional information customised for a particular therapeutic situation. There should be sufficient emphasis and specific advice about the likelihood of reaction recurrence and what to do should it occur. Patients and their families should be educated about early symptoms of possible adverse reactions and action required e.g. to promptly seek medical advice for certain prodromes of expected adverse reactions.

The proposed algorithm is meant to aid the thought process, stimulate further debate as further guidance on specific situations may well be required. These algorithms might initially be conservative and restrictive. However, as safety evidence from unintentional and intentional rechallenge accumulates based on the use of such algorithms, then the threshold for rechallenge may be lowered and the algorithm appropriately modified. Both positive and negative rechallenge situations add valuable information and companies should seek additional data from medical queries in all cases in order to generate a body of evidence about real-life use of a medicine.

In the light of the renewed emphasis on deterring of medication errors and off-label use in the European pharmacovigilance legislation, applying these regulations must not inadvertently interfere with responsible prescribing of essential medicines. The worry is that such use may, in a regulatory sense, be viewed as medication error, misuse or off-label use. Hence, intentional rechallenge as a result of thoughtful deliberation of benefit/risk should be differentiated from accidental rechallenge and a medication error, even if the rechallenge ultimately proves to be harmful.

In conclusion, each significant adverse reaction potentially leading to treatment discontinuation should have a reaction-specific rechallenge algorithm. Evidence-based risk assessment and minimisation measures should be proposed as a collaborative effort by drug manufacturers, expert centres or professional societies. All such focussed activities should be addressed as part of active risk management planning. However, ultimately the ethical and safety responsibility rests with the individual prescribers and patients (102).

Figure 7: Points to consider in clinical decision making in the setting of intentional rechallenge



8 CONCLUSION

The findings in this work indicate that a certain level of prediction of ADRs is possible based on pre-marketing clinical and non-clinical data. At the same time, there is apparently insufficient effort placed on risk prediction and mitigation during drug development. The effort of drug manufacturers is understandably focussed on demonstration of safety and efficacy overall. Patients at risk of developing ADRs are often excluded from development programs due to risk of liability, but in real-life they equally require treatment.

As demonstrated by the Kaplan-Meier analysis, risk management planning may lead to earlier detection of safety concerns. Time to detection of an important safety finding is of utmost importance to public health. Earlier detection of safety concerns leads to decreased morbidity, mortality due ADRs and hence a range of benefits to healthcare. At the same time, the application of Kaplan-Meier analysis has been extended to the field of risk management planning; a field in which it has not been traditionally used.

Predicting ADRs is particularly important in high-risk situations in which the expected benefit is also high. Rechallenge following an ADR is such an example in which the benefit may, or may not, justify the risk. Rechallenge is of importance in all pharmacotherapy and therapeutic risk management. However, in immunological ADRs to biopharmaceuticals, rechallenge has particular risk-predictive features. It is proposed that each significant adverse reaction potentially leading to treatment discontinuation should have a reaction-specific rechallenge algorithm.

As in all pharmacotherapy and medical interventions in general, the expected benefits must outweigh the expected risk. Risk management for biopharmaceuticals is achieved by two principal means. The first step is risk estimation or prediction. This includes the measures taken to reliably assess risk factors of immunogenicity in a particular patient. The second step is risk mitigation, which covers the measures taken to decrease the risk to the minimum.

Pharmacogenomics showed success in estimating the likelihood of adverse reactions to several small molecule drugs. Several immunogenicity assessment tests have been evaluated, but better predictive factors still need to be established for biopharmaceuticals.

Risk of an ADR is particularly high when an ADR (or suspected ADR) has already occurred and the treatment should be resumed. Patients and populations at risk should be identified as

part of risk-management planning. For biopharmaceuticals more than for other drugs, rechallenge following ADRs is a major challenge. The set of predictive methods should be particularly elaborate for such situations. A detailed algorithm for assessment of immune response tailored to each individual biopharmaceutical should describe the conditions under which benefit outweighs the risk.

Developmental (pre-registrational) as well as established post-marketing risk management and minimisation action plans should routinely address both predictive methods and mitigation of consequences of biopharmaceutical immunogenicity starting from early development. The assay battery used in pre marketing should form a package with the drug; therefore. This is in accordance with the current requirement in which an RMP is approved along with a drug.

Risk management plans are now provided as open access to the public, including prescribers, pharmacists and patients. However, RMPs are still a document applicable primarily to the industry and regulators, and have not truly entered clinical/pharmacy practice. Early identification of risk on individual patient level is relevant at the individual's, as well as on the global level. Involvement of patients in their own healthcare is growing. They are key stakeholders contributing to safety and, last but not least, pharmacoeconomics of early identification of risks.

9 SUMMARY

Pharmacovigilance is changing. It is no longer a passive discipline of awaiting and detecting adverse reactions, but active in predicting and managing risks. Pharmacovigilance of biopharmaceuticals deals with all the complexities of conventional small molecule drugs, and on top of that, takes into account its own specificities. Most notably, this is immunogenicity. For biopharmaceuticals the task is, therefore, multiple-fold more complex. Medical product database search for adverse drug reactions to biopharmaceuticals was performed on the US FDA Safety Information and Adverse Event Reporting Program (FDA Medwatch). The search focused on safety alerts for monoclonal antibody therapeutics. Kaplan Meier analysis of time to Medwatch safety alert was also applied, in which the time to safety alert was used as time to event i.e. "survival".

The findings in this work indicate that a certain level of prediction of ADRs is possible based on observed pre-marketing clinical and non-clinical data. In addition to the actually observed ADRs, a large percentage can be predicted based on drug structure and the drug target (i.e. mechanism of action and potential adverse reaction). Expanding the spectrum of in vitro and in vivo predictive tests and their application in routine clinical use could contribute further to predictability assessment.

As demonstrated by the Kaplan-Meier analysis, risk management planning may lead to earlier detection of safety concerns. Time to detection of an important safety finding is of utmost importance to public health. Earlier detection of safety concerns leads to decreased morbidity and mortality due ADRs and hence a range of benefits to healthcare.

Predicting ADRs is particularly important in high-risk situations in which the expected benefit is also high. Rechallenge following an ADR is such an example in which the benefit may, or may not, justify the risk. Rechallenge is of importance in all pharmacotherapy and therapeutic risk management. However, in immunological ADRs to biotherapeutics, rechallenge has particular risk-predictive features. Each significant adverse reaction potentially leading to treatment discontinuation should have a reaction-specific rechallenge algorithm. A sample generic algorithm is proposed in this thesis which should be adapted to each particular high-risk high-benefit situation.

10 ÖSSZEFOGLALÁS

A farmakovigilancia változik, ma már nemcsak egy tudomány, mely passzívan várja és felismeri a nemkívánatos hatásokat, hanem aktívan előrelátóan keresi és kezeli a kockázatokat. A biotechnológiával készült gyógyszerek farmakovigilanciája a hagyományos kismolekulájú gyógyszerek minden nehézségével foglalkozik, és ezen felül figyelembe veszi azok speciális sajátosságait, köztük az immunogenitást. A biotechnológiával készült gyógyszerek nemkívánatos gyógyszerhatásairól az amerikai FDA Medwatch Programjában végeztek kutatást, amely a monoklonális antitestekre adott biztonságossági riasztásokra fókuszált. A Medwatch biztonságossági riasztásokhoz a Kaplan-Meier féle időanalízist alkalmaztam, ahol az idő a biztonságossági riasztáshoz az eseményig eltelt időt jelenti, ebben az esetben a „túlélést”.

A kutatás eredményei azt mutatják, hogy a nemkívánatos gyógyszerhatások bizonyos fajta előrejelzése lehetséges a megfigyelt forgalomba hozatal előtti klinikai és nem-klinikai adatok alapján. A már megfigyelt nemkívánatos gyógyszerhatásokon kívül, egy nagyobb százalék előre jelezhető a gyógyszer szerkezete és a gyógyszer célpontja alapján (a gyógyszer hatásmechanizmusa és lehetséges nemkívánatos hatások). Az *in vitro* és *in vivo* prediktív tesztek kiszélesítése és ezek rutin klinikai használata hozzájárulhat a további kiszámíthatósági vizsgálatokhoz. A Kaplan-Meier analízis eredménye alapján, egy kockázatkezelési terv segíthet a biztonságossági kockázat korai felismerésében. Egy biztonságossági kockázat felismeréséig eltelt idő közegészségügyileg rendkívül fontos. A biztonsági kockázatok korai felismerése csökkenti a nemkívánatos gyógyszerhatások által okozott morbiditást és mortalitást, ezáltal számos előnnyel jár az egészségügynek.

A nemkívánatos gyógyszerhatások előrejelzése különösen fontos magas rizikójú esetekben, ahol a várható előny szintén magas. Egy nemkívánatos gyógyszerhatás újbóli megismétlődése (rechallenge) egy jó példa arra, amikor az előny nem indokolja a kockázatot. A megismétlődés fontos minden gyógyszeres terápiában és terápiás kockázatkezelésben, azonban a biotechnológiával készült gyógyszereknél kialakult immunológiai nemkívánatos gyógyszerhatások esetében az újbóli megismétlődésnek sajátos kockázatjósló funkciói vannak. Minden egyes jelentős nemkívánatos hatásnak, mely potenciálisan a kezelés megszakításához vezet, van egy reakció specifikus kiújulási algoritmusa. Ebben az esetben egy olyan mintán alapuló algoritmus alkalmazását javasolom, amelyet minden egyes magas-kockázatú, magas-előnyű esethez igazítani kell.

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12 OWN PUBLICATIONS

Own publications related to the thesis

- Stanulović V, Venegoni M, Edwards B. (2013) Intentional Rechallenge: Does the Benefit Outweigh the Risk? Drug Saf. 36(3):155-61.
- Stanulović V, Zelko R, Kerpel-Fronius S. (2011) Predictability of Serious Adverse Reaction Alerts for Monoclonal Antibodies. Int J Clin Pharm Ther. 49(3):185-90.

Own publications not directly related to the thesis

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