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Review

Immunotoxicity of monoclonal antibodies

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Monoclonal antibodies (mAbs) are large molecules intended to bind to specific targets often expressed on the immune system, and to treat various immunopathological conditions. Therefore, mAbs can be considered to have a high potential for immunotoxicity, which is reflected in the clinical experience accumulated on mAbs-induced adverse effects related to immunosuppression, immunostimulation and hypersensitivity (immunogenicity). So far, non clinical immunotoxicity studies have been inadequate to address all safety issues in relation to the possible immunotoxicity of mAbs, because they are fraught with limitations and pitfalls primarily related to the lack of relevant animal species. In addition, clinical studies rarely include validated end-points dedicated to the prediction of immunotoxicity. With the ongoing development of mAbs as novel therapeutic strategies for a wide variety of diseases, efforts should be paid to improve our understanding of mAbs-induced immunotoxic effects and design dedicated strategies to assess their immunological safety, both non clinically and clinically.

Introduction

Since the seminal paper that Köhler and Milstein published in 1975,[1] showing that a line of murine myeloma cells can be fused with healthy antibody producing B cells to generate one single exquisite type of antibody against a target antigen, i.e., a monoclonal antibody (mAb), tremendous progress has been made to develop mAbs applicable to the diagnosis or treatment of various pathological conditions. To date, at least 20 mAbs have been approved by the US FDA and still more have been approved worldwide. It can be estimated that several hundreds of candidate mAbs are under current development.[2]

The clinical use of mAbs as a novel class of therapeutic agents has been rapidly expanding to include a wide range of pathological conditions, such as graft rejection, cancer, auto-immune diseases, asthma, selected infections, cardiac ischemia… To overcome the limitations and pitfalls of early marketed mAbs, tremendous efforts have been made to improve efficacy or pharmacokinetics, and reduce immunogenicity. Over the years, the clinical use of mAbs has been associated with the development of a variety of adverse effects in human patients among which those involving the immune system can be considered to be the most frequent, if not the most significant clinically.

This paper is an overview of the immune-mediated adverse effects of mAbs in treated human patients, and current non clinical and clinical approaches for the safety evaluation of mAbs with specific reference to immunotoxicity. Even though the majority, if not all available guidelines dealing with the immunotoxicity evaluation of medicinal products are focused on immunosuppression, a term often misleadingly used as synonymous to immunotoxicity, it is essential to keep in mind that the scope of immunotoxicology actually covers four different aspects: immunosuppression, immunostimulation, hypersensitivity and auto-immunity.[3] Indeed, each aspect or category of immunotoxic effects is characterized by distinct clinical adverse consequences and requires dedicated animal models and assays to be assessed during safety studies.

Clinical Immunotoxic Effects of mAbs

Immunosuppression-related adverse effects of mAbs. Adverse effects related to immunosuppression. It has long been recognized that treatments with potent immunosuppressive agents can be associated with more frequent, and often more severe and relapsing infections.[4] Overall, no single pathogen is specifically involved in infectious complications associated with immunosuppression, and all types of pathogens including bacteria, viruses, fungi and parasites can be encountered. Infections of the respiratory and gastrointestinal tracts are more frequent, but the central nervous system and the skin are also affected. In addition, some infections can be atypical or opportunistic, and then characterized by involvement of pathogens, e.g., *Pneumocystis carinii* that normally do not induce overt infections in fully immunocompetent human beings, or by their localization at uncommon sites, e.g., brain abscess in aspergillosis or toxoplasmosis. Finally, infectious complications of (moderately) immunosuppressive drugs can be clinically and microbiologically unremarkable so that an increased incidence of these infections can only be detected if dedicated (pharmaco) epidemiological studies are conducted in treated human subjects.

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Another type of major adverse effects associated with immunosuppressive drug therapy is the occurrence of virus-induced neoplasias.[5] Many retrospective and prospective studies evidenced a greater risk (up to 50-fold) of lymphoproliferative disorders—primarily B lymphomas—in organ transplant patients. Although lymphoproliferative disorders attracted much attention, other virus-induced cancers, such as skin cancers, cancers of the lips and Kaposi sarcomas may actually be more frequent.

Infectious complications and mAbs. Quite a few mAbs have been or are being developed to exert immunosuppressive effects useful for the treatment of various conditions, such as the prevention of graft rejection, or auto-immune diseases including rheumatoid arthritis, Crohn disease or multiple sclerosis. In addition, mAbs are increasingly used as anti-cancer agents and these mAbs can also exert unintended immunosuppressive effects.

To date, the most extensive clinical experience accumulated with immunosuppressive mAbs is related to anti-TNF drugs. By targeting TNFα receptors, these drugs decrease elevated TNFα levels either systemically or at inflammation sites, and dramatically alleviate clinical signs and symptoms in chronic inflammatory diseases. The anti-TNF mAbs infliximab, adalimumab and certolizumab pegol have been approved for the treatment of rheumatoid arthritis and/or Crohn disease, and several others are under clinical development. Because TNFα also plays a critical role in the host's defense against a variety of bacterial and viral pathogens, infectious complications have been observed in human patients treated with anti-TNF drugs including anti-TNF mAbs.[6,7] Following the first report of 70 cases of tuberculosis in infliximab-treated patients recorded by the US FDA Adverse Event Reporting System,[8] tuberculosis has been observed as a complication of therapy with every anti-TNF drug, although the risk is considered to be lower in patients treated with etanercept, a recombinant dimeric soluble TNFα receptor protein, than with either infliximab or adalimumab.[7] Reactivation of latent tuberculosis is thought to play a key role and various institutions have released recommendations, which led to a markedly decreased incidence of tuberculosis in patients treated with anti-TNF mAbs.[9] Even though tuberculosis is a major safety concern, other infectious complications including bacterial and fungal opportunistic infections, such as histoplasmosis, listeriosis, aspergillosis, candidiasis, *Pneumocystis carinii* pneumonia and coccidioidomycosis have also been reported in association of anti-TNF mAbs.[10-12] Patients treated with anti-TNF drugs/mAbs are also suspected to have an increased risk of respiratory tract, skin, urinary tract and bone and joint infections.[7]

Infections have been reported in human patients treated with a number of other mAbs, and the incidence of associated infectious complications is generally in keeping with their mechanism of action.[13] However, it is noteworthy that infectious complications are usually less frequent and/or less severe in patients treated with mAbs than in those treated with primary immunosuppressive agents. Although bacterial or viral infections have been noted in up to 30% of patients with B lymphoma or auto-immune disease treated with rituximab, a chimeric anti-CD20 mAb, severe or opportunistic infections were rather infrequent, which is in agreement with conserved T cell functions in rituximab-treated patients.[14] From 30 to 97% of patients with chronic lymphocytic leukemia treated with alemtuzumab, a humanized IgG1 mAb that targets the CD52 antigen and produces profound cellular immune dysfunction, developed infectious complications including severe and sometimes lethal infections, such as disseminated viral infections, systemic Candida infections, tuberculosis reactivation and invasive fungal infections.[15] In contrast, breast cancer patients treated with trastuzumab, a humanized mAb targeting the human epidermal growth factor receptor-2, or patients treated with either palivizumab, a humanized mAb directed against the human respiratory syncytial virus (HRSV) fusion glycoprotein, or omalizumab, a humanized anti-IgE mAb, developed only infrequent and unremarkable infections.[3]

The suspected parallel between the development of infectious complications and the mechanism of action of mAbs proved to be somewhat more complicated by the occurrence of progressive multifocal leukoencephalopathy (PML) in three multiple sclerosis patients treated with natalizumab, a humanized mAb that targets α4 integrin and thus prevents the entry of inflammatory cells into

Table 1 The four categories of immunotoxic effects and associated adverse clinical consequences

Immunosuppression	Immunostimulation	Hypersensitivity	Autoimmunity
Infectious complications (*)	Acute reactions related to cytokine release (*)	Anaphylaxis (*)	Systemic autoimmune reactions (*)
Virus-induced neoplasias (*)	More frequent auto-immune diseases (*)	Immune-complex mediated reactions (*)	Organ specific autoimmune reactions
	More frequent allergies to unrelated allergens		
	Inhibition of CYP450-dependent pathways		

Clinical consequences previously described with mAbs are denoted (*).

Table 2 Main guidelines specifically applicable to the non-clinical safety evaluation of mAbs

ICH S6 Guideline on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (1997; revision ongoing)
US FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997)
EMEA Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (2007)
EMEA Guideline on the Strategies to Identify and Mitigate Risks for First-in-Human Clinical Trials with Investigational Medicinal Products (2007)

tissues.[16] PML is a rare disease associated with immunosuppression involving T cell functions, and the JC virus is considered to be the causative agent. So far, no definitive explanation has been provided to establish whether and how natalizumab could trigger PML in treated patients.

Virus-induced neoplasia and mAbs. Although virus-induced neoplasias have sometimes been described in patients treated with mAbs, their incidence is much less than infectious complications. This may be due to the fact that virus-induced neoplasias preferentially develop in profoundly immunocompromised patients, a situation that is not commonly encountered in patients chronically treated with anti-TNF mAbs that are moderately immunosuppressive, or in patients treated with markedly immunosuppressive anti-cancer mAbs, but typically for rather short periods of time. Thus, rare case reports of lymphoma or skin cancer in patients treated with anti-TNFα mAbs have been published, and recent studies evidenced a lack of convincing association.[17,18]

These clinical findings highlight the lower potential of most currently available mAbs to induce frequent and/or severe immunosuppression-related adverse effects as compared to immunosuppressive agents, such as those used in the long-term prevention of organ transplant rejection. Indeed, most mAbs are typically intended to produce targeted immune downmodulation, whereas potent immunosuppressive agents induce global immunosuppression.

Immunostimulation-related adverse effects of mAbs. Adverse effects related to immunostimulation. In sharp contrast to immunosuppression, adverse effects related of immunostimulation have been recognized only recently with the introduction of recombinant cytokines, such as rIL-2 and the interferons (IFNα/β) in the clinic. At least four types of adverse effects have been described in relation to immunostimulation including cytokine release-associated acute reactions, more frequent autoimmune diseases and hypersensitivity reactions to unrelated allergens, and inhibition of CYP450-dependent biotransformation pathways.[4]

Cytokine release-associated acute reactions and mAbs. Cytokine release has been a matter of major concern since the highly publicized report of a so-called "cytokine storm" that developed on 13th March 2006 in six healthy human volunteers administered TGN1412, an anti-CD28 superagonistic mAb during a phase I clinical trial.[19] In fact, most, if not all of our current understanding of these reactions was available before this dramatic event occurred.[20]

Cytokine release-associated acute reactions consist of clinical manifestations ranging from flu-like reactions to cytokine release syndromes. Flu-like reactions are typically characterized by mild to moderate fever (38–39.5°C) associated with chills, fatigue, myalgias, headache and/or nausea that usually abate within a few hours. Alleviation or prevention can be easily obtained with administration of paracetamol (acetaminophen) or a non-steroidal anti-inflammatory drug, such as ibuprofen. In some instances, the reaction is more severe and often dose- or treatment-limiting, and is then referred to as a cytokine release syndrome, which is characterized by marked hyperpyrexia (>40°C) associated with cardiovascular disturbances, including a drop in blood pressure, or even cardiovascular collapse potentially leading to cardiac ischemia, and various neurological disorders, such as tremor, rigor, confusion, obnubilation or seizures. A cytokine storm is characterized by similarly severe clinical manifestations associated with multi-organ failure primarily affecting the lung (severe acute respiratory syndrome or SARS) and the kidney. To the best of our knowledge, cytokine storm, a common finding in avian flu, has only been reported once in association with mAbs (i.e., the TGN1412 acute episode).

Whatever the severity of the reaction or the term used to describe it, the release of pro-inflammatory cytokines including IL-1β, TNFα, IFNβ, IFNγ, IL-6 and IL-8 is considered to be the main underlying mechanism.[20] Cytokine release is the consequence of a direct activation of various immunocompetent cells including macrophages, monocytes, lymphocytes and NK cells. Depending on the intrinsic properties of the compound, activation may affect one or several cell populations with variable intensity, and thus result in a variable severity of clinical manifestations.[21]

As early as 1989, the first dose of muromonab (OKT3) was shown to cause hyperpyrexia, chills, tremor, nausea, vomiting and diarrhea, joint pains and hypotension in approximately 50% of patients.[22] The symptoms were associated with a sharp increase in TNFα and IFNγ concentrations suggesting the involvement of cytokine release. Since then, acute adverse reactions that are alternatively depicted as cytokine release syndromes or acute infusion reactions have been described in relation to the administration of a number of mAbs.[23] The highest incidence of infusion reactions was observed with rituximab and trastuzumab: severe, but usually reversible reactions were reported in approximately 10% of patients. Approximately 80% of fatal reactions were associated with the first infusion of rituximab. In contrast, the first infusion of trastuzumab produced mostly mild to moderate reactions in approximately 40% of patients, and only infrequently during subsequent infusions. Reactions to the first infusion of cetuximab were usually mild to moderate affecting 12–19% of patients, while severe reactions were seen in 3% of patients and were fatal in 1 of 1,000. Finally, infusion reactions occurred in 4% of patients given a first dose of panitumumab and were severe in 1%.

The mechanism of acute infusion reactions following injection of mAbs is not fully elucidated. Although cytokine release is considered to play a pivotal role, the somewhat different clinical features in most mAb-associated infusion reactions as compared to flu-like reactions that are commonly seen following administration of immunomodulatory recombinant cytokines, suggest the involvement of additional mechanisms, such as activation of the complement cascade as evidenced in acute infusion reactions caused by rituximab.[24] An antigen-specific (IgE-mediated) hypersensitivity reaction can be easily ruled out after injection of the first dose of a mAb due to the lack of the absolutely required prior sensitization, but a convincing diagnosis may be more difficult to attain after repeated administrations.

More frequent autoimmune diseases and mAbs. Treatments with immunostimulatory agents have long ago been suggested to be associated with more frequent auto-immune diseases, a hypothesis confirmed rather recently following the introduction

of recombinant therapeutic cytokines.[3] Indeed, patients treated with either rIL-2 or IFNα have been shown to develop various auto-immune diseases that were identical to the spontaneous diseases, but seemingly more frequent.

It is critical to carefully differentiate more frequent auto-immune diseases that may be caused by immunostimulating or immunomodulating agents from drug-induced auto-immune reactions as any type of auto-immune disease clinically and serologically identical to a spontaneously developing disease is suspected to be more frequent as a consequence of the facilitating, even though ill-understood role of immunostimulation, whereas systemic drug-induced auto-immune reactions typically bear major clinical and serological differences with their spontaneous counterpart, e.g., drug-induced lupus vs systemic lupus erythematosus, and a particular drug usually triggers only one given type of auto-immune reaction.

As regards mAbs, more frequent auto-immune diseases have been described in patients treated with anti-TNF drugs including anti-TNF mAbs. Recently, the analysis of 53 published case reports led to the conclusion that in most instances the diagnosis of drug-induced lupus would be excluded if the American College of Rheumatology criteria were strictly applied.[25] This suggests that lupus associated with anti-TNF mAbs is not a drug-induced auto-immune reaction, but presumably a consequence of their immunomodulating properties, even though the underlying mechanism is not known.

More frequent hypersensitivity reactions to unrelated allergens and mAbs. Even though it might be logical to suspect that immunostimulation could well result in enhancing normal as well as abnormal immune responses, such as asthma, urticaria or hay fever triggered by food or environmental allergens, extremely few clinical data have been provided to substantiate this suspicion.[4] Importantly, these reactions must be clearly distinguished from drug-induced hypersensitivity reactions ("drug allergies").

However, several controlled clinical studies provided compelling evidence that hypersensitivity reactions to radiological contrast media are significantly more frequent in cancer patients treated with rIL-2 than in matched untreated patients.[3] To the best of our knowledge, similar findings have not been reported with mAbs.

Inhibition of CYP450-dependent biotransformation pathways and mAbs. There is a large body of experimental evidence that immunostimulating agents can inhibit CYP450-dependent biotransformation pathways via downregulation of major isoforms at the level of gene transcription involving IL-1α, IL-1β, IL-2β, IL-6, TNFα, IFNα, IFNγ and TGFβ. The administration of recombinant cytokines including rIL2, rIL-10, IFNsα or β has been shown to induce significant changes in the pharmacokinetics of several combined drugs in human patients.[3] However, no such findings have seemingly yet been reported with mAbs.

Mabs-induced hypersensitivity reactions. Immunogenicity is a major safety concern as regards mAbs. It has long been recognized that molecules of sufficient size and foreign, "non self" origin can act as direct immunogens and thus induce the production of specific antibodies that subsequently react with small bits of the eliciting macromolecule (the so-called epitopes). All currently available or publicly known mAbs can be considered to be potential direct immunogens as their molecular size is large enough, and their structure different from endogenous proteins. Despite current efforts to produce highly humanized or "human-like" mAbs, immunogenicity is not yet totally eradicated.[26] The immunogenic potential of mAbs ranges from non immunogenic—where no antibodies are generated—to strongly immunogenic—where most treated subjects develop specific antibodies. Clinical consequences range from a lack of overt consequences to life-threatening complications. It is beyond the scope of this paper to review the factors shown to affect the immunogenic potential of therapeutic proteins including mAbs.[27,28]

Treatment of human patients with mAbs can indeed be associated with the development of specific antibodies. As already mentioned, the incidence of patients developing specific antibodies, the quantity generated (as reflected by serum levels), and the clinical relevance of these antibodies are extremely variable, and as of today, impossible to anticipate from the results of preclinical safety studies. Therefore, the search for specific antibodies in the sera of human subjects enrolled in clinical trials is a critical step in the evaluation of the immunogenicity of any novel mAb.[29] In a number of patients, specific antibodies are inconsequential. Neutralizing antibodies can also be detected using bioassays or cell-based assays.[30] They can block the biological activity either by binding directly to epitope(s) within the active site, or by steric hindrance due to binding to epitope(s) in close proximity to the active site. The presence of neutralizing antibodies may not result in a clinical effect, but a decrease in efficacy may require administration of higher doses.

The presence of specific antibodies to mAbs is seldom associated with immuno-allergic hypersensitivity reactions. Rare anaphylactic reactions associated with mAbs including cetuximab,[31] infliximab,[32,33] basiliximab[34] or abciximab[35] have been reported. As already mentioned, it is essential, in sharp contrast to quite a few misleading case reports in the literature, to differentiate acute infusion reactions that can occur during the first infusion of a mAb, from anaphylactic shock than can only occur following a re-administration of the same mAb. Because of the presence of specific antibodies in their sera, treated patients are suspected to be at risk of developing serum sickness (type III hypersensitivity). In fact, only a small percentage of infliximab- and rituximab-induced infusion reactions are delayed and thus could involve a type III mechanism. One documented case of serum sickness has been reported following infliximab administration.[36]

Mabs-induced auto-immune reactions. More frequent auto-immune diseases caused by immunostimulating or immunomodulating agents must be differentiated from drug-induced auto-immune reactions. So far, no drug-induced auto-immune reaction has been reported in association with mAb treatment.

Immunotoxicity Evaluation of mAbs

The accumulated clinical experience on adverse effects associated with mAb treatments indicates that only limited information has been gained from non clinical (preclinical) safety studies in the past as quite a few adverse effects observed in treated patients were

indeed not expected from the results of prior non clinical studies. It should, however, be emphasized that the non clinical safety evaluation of therapeutic proteins including mAbs is fraught with many limitations and pitfalls as extensively discussed elsewhere.[37-39]

The selection of a relevant animal species is undoubtedly a major limitation.[40] To reduce or even eliminate the risk of immunogenicity in humans, efforts are being paid to produce increasingly human-like mAbs,[41] but this in turn results in their increased immunogenicity in laboratory animals as evidenced by the development of neutralizing antibodies that tend to reduce the duration and thus the predictivity of non clinical safety studies, or by the occurrence of immune-mediated adverse events, e.g., anaphylaxis or immune complex-mediated reactions, that are not relevant to man. Moreover, the targets of newly developed mAbs are increasingly human-specific and this likewise results in lesser relevance of conventional animal models. From the perspective of immunotoxicity evaluation, another characteristic feature of many mAbs is that they are intended to target specific components of the immune system, which highlights the need for a careful and adequate evaluation of the immunotoxicity potential of mAbs.

Non clinical immunotoxicity evaluation of mAbs. ICH guideline S6 on the preclinical safety evaluation of biotechnology-derived pharmaceuticals[42] is the only guideline available to orient the non clinical immunotoxicity evaluation of mAbs, even though this is a very broad guideline with no specific focus on mAbs. Revision of this guideline is ongoing, but no significant changes as regards immunotoxicity studies seem to be expected. It is noteworthy that ICH guideline S6 was the first guideline to stress the need for an immunotoxicological evaluation of at least one broad group of medicinal products, and also that ICH guideline S8 on immunotoxicity studies for human pharmaceuticals does not apply to biotechnology-derived products and other biologicals.[43] That immunogenicity as well as suppression or stimulation of the immune system were addressed is another important contribution of ICH guideline S6. Unfortunately, no recommendation or suggestion was made in this guideline to assist in the selection of an adequate evaluation strategy.

Immunosuppression. Because immunotoxicologists used to focus their attention on immunosuppression, quite a few animal models and assays have been standardized and validated to reasonably predict the unexpected or unintended immunosuppressive effects of candidate drugs.[44] Even though ICH guideline S8 is overtly said not to apply to the non clinical immunotoxicity evaluation of biotechnology-derived products including mAbs, several recommendations can nevertheless be used in this context.

Standard toxicity studies can provide useful information including clinical signs, such as infections or tumors, hematological changes affecting leukocyte, neutrophil or lymphocyte counts, and histopathological findings in the thymus, spleen, main lymphoid organs and Peyer's plaques. Although ICH guideline S8 follows a weight of evidence approach, which means than no immune function assay is required systematically, but only when a cause for concern exists, which is reminiscent of the tiered immunotoxicity testing strategy,[45] ICH guideline 6 states that such a tiered strategy is not recommended. In fact, as many mAbs target specific components of the immune system, the weight of evidence approach would suggest that additional immunotoxicity testing should be considered.

There is a wide consensus among immunotoxicologists and regulators in the field of medicinal products as well as other areas, such as agrochemicals and medical devices, that the first-line assay to be considered for assessing the immunosuppressive potential of a new molecular entity is a T-dependent antibody response (TDAR) assay.[46] The anti-sheep red blood cell plaque-forming cell (PFC) assay[47] and the anti-keyhole limpet hemocyanin (KLH) antibody ELISA[48] are the main TDAR assays. The PFC assay has long been used in the context of immunotoxicity evaluation so that an extremely large database of published results on many medicinal or chemical products is available. However, this assay does not use a well-standardized antigen, can only be applied to rodents, is time-consuming, and finally the results are based on a rather subjective assessment and cannot be automated. In contrast, KLH is a well-standardized antigen, the ELISA technique can be easily automated and the anti-KLH ELISA can be used in all mammal species including non rodents. Thus, the anti-KLH ELISA is nowadays widely promoted as the preferred TDAR assay. It is nevertheless critical to bear in mind that this assay is not adequately standardized with significant inter-laboratory variations in the dose, route of administration and number of injections, and extremely poorly validated. A reasonable amount of data is available in the rat, but these data are limited to few potent, immunosuppressive drugs, such as cyclosporine and cyclophosphamide, whereas data in other species are extremely scarce (dog, monkey) or lacking (mouse). This obvious lack of adequate standardization and validation probably accounts for the reluctance of regulators outside the medicines area to recommend the anti-KLH ELISA.

Among a long list of possible immune function assays, lymphocyte proliferation assays to measure cell-mediated immunity and flow cytometry-based assays of phagocytosis can also be used. If results obtained during standard toxicity studies and in a TDAR assay suggest a cause for concern as related to potential immunosuppression, other assays are to be used case by case. One of these assays is lymphocyte subset analysis. Typically, the analysis is restricted to total B and T lymphocytes, $CD4^+$ and $CD8^+$ lymphocytes, and possibly natural killer (NK) cells and monocytes.[44] It is noteworthy that lymphocyte subset analysis is not an immune function assay. The use of lymphocyte activation markers would be more instructive, but none has been clearly shown to predict immunotoxicity. However, changes in lymphocyte subsets can be used as a biomarker, ensuring needed transitions across animal species and from animal to man. A number of other immune function assays can be used, such as the measurement of NK cell activity using either a ^{51}Cr-release assay or flow cytometry, but its relevance for immunotoxicity evaluation is debatable.[49]

As already mentioned, mAbs are increasingly human-specific, either because they are humanized to reduce immunogenicity or because they are directed towards highly specific human targets. Thus, non human primates are often the only relevant species available for non clinical immunotoxicity evaluation. Unfortunately,

only a few monkey-specific reagents are commercially available and relatively little is known of the normal immune system or immunopathological responses in monkeys, which is a major limitation to ensure sufficient predictability of non clinical immunotoxicity studies in monkeys. In addition, many mAbs exert intended or unintended pharmacodynamic effects on the immune system so that the development of dedicated safety immunopharmacology studies, especially in non human primates can be expected to improve the predictability of non-clinical immunological safety studies. Finally, transgenic animal models are an interesting alternative, but current models lack sufficient standardization and validation. Therefore, research efforts should be directed to design, standardize and validate new models and assays applicable to immunotoxicity safety evaluation, in particular as regards mAbs.

Immunostimulation. The development of recombinant therapeutic cytokines showed that immunostimulation can result in adverse effects and this also applies to mAbs that can trigger untoward activation of the immune system with a variety of adverse consequences in non-clinical safety studies.[50] In sharp contrast to immunosuppression, no well-defined strategy to predict enhanced immune response following treatment with medicinal products is available.[44] Assessment of the same endpoints—either functional or not—to predict immunosuppression and immunostimulation is sometimes recommended, but so far no extensive results support (or deny) the validity of this approach.

Predicting the cytokine releasing properties of mAbs is a major safety concern. The TGN1412 episode showed that reliable prediction in non clinical studies can be hard to obtain.[51] Indeed, non human primates are not good predictors of cytokine release syndromes. It is noteworthy that clinically significant cytokine release syndromes have seemingly not been reported in monkeys including chimpanzees treated with mAbs, and their measured cytokine release is consistently less than in humans. Therefore, in vitro assays using human cell lines may be an alternative to in vivo monkey studies, but wide inter-laboratory variations are major limitations at the present time. In addition, there is no extensive database comparing the cytokine releasing properties of a large panel of therapeutic proteins including mAbs in in vitro assays to the magnitude of cytokine release-associated clinical reactions.

The prediction of other adverse consequences of immunostimulation, such as more frequent auto-immune diseases or hypersensitivity reactions to unrelated allergens is hampered by the lack of adequate animal models, especially in non human primates.

Hypersensitivity. The immunogenicity of mAbs is a major safety issue, but the predictivity of animal models for evaluation of immunogenicity is low as clearly stated in the recent EMEA guideline on the immunogenicity assessment of biotechnology-derived therapeutic proteins.[52] With the exception of comparing closely related products (e.g., biosimilars), currently available animal models cannot be expected to provide relevant information. As previously mentioned, the development of anaphylaxis or other immuno-allergic reaction in animals treated with mAbs can be considered of very limited, if any relevance for humans. In contrast, pseudo-allergic reactions due to a non antigen-specific release of mediators, such as histamine or anaphylatoxins, the by-products of activation of the complement cascade can be assessed either ex vivo or in vitro.

Auto-immunity. The potential for any medicinal product including mAbs to induce auto-immune reactions in humans is beyond reach of current non clinical immunotoxicity evaluation.

Clinical immunotoxicity evaluation of mAbs. Clinical immunotoxicology is the missing link in the immunological safety evaluation of all medicinal products.[53] This is particularly obvious for mAbs because of the many limitations and pitfalls in the prediction of their immunotoxic potential during non clinical studies. The transition from animal to human studies and the expectations of clinicians to improve the immunological safety evaluation of drug candidates is a key issue nowadays.

There is an urgent need to identify relevant biomarkers of immunotoxicity to be used in clinical trials, and not only immunological markers of efficacy. Ideally, these biomarkers, or at least some of these, should also be applicable to animal safety studies to ensure the needed transition from animal to man. As a wide variety of immunological endpoints or immune function tests are routinely used by clinical immunologists for the diagnosis of various immunopathological conditions including primary or secondary (acquired) immunodeficiencies, hypersensitivity reactions and autoimmune diseases,[54] it is recommended to assess which of these endpoints or immune function tests could be used as biomarkers during clinical trials to improve the immunologic safety evaluation of mAbs. This effort is likely to require a multi-disciplinary approach involving immunologists, clinical pharmacologists and immunotoxicologists to ensure that the selected biomarkers are adequately standardized and validated as well as cost-effective to be subsequently recommended for use during clinical trials. The search for novel human biomarkers of immunotoxicity is also a priority. The rapid development of new technologies, such as multi-parameter flow cytometry, cellular imaging or (immuno) toxicogenomics offers a wealth of possibilities that deserve careful consideration and focused evaluation.

However, the design, standardization and validation of relevant human biomarkers of immunotoxicity will require dedicated efforts and cannot be expected to change the scene in the short term. In the meantime, current protocols of clinical trials could be improved to ensure a better evaluation of the immunological safety of mAbs. As reviewed above, the majority of adverse effects on the immune system potentially associated with mAbs can be suspected to occur, at least from a theoretical point of view, based on the available information on the mechanism of action, intended or unintended targets and structure in a broad sense. These suspected causes for concern could be addressed more thoroughly during clinical trials. One example is the possible occurrence of infectious complications. The detection of infections complications can be based on their respective incidence in treated and control patients. However, a detailed clinical, paraclinical and microbiological diagnosis whose procedure is clearly described in the clinical trial protocol will help identify whether uncommon pathogens, uncommon clinical signs or other uncommon findings are more

frequent in treated patients, which can therefore give clues for a better immunotoxicity risk assessment. The same approach could be applied to the detailed diagnosis of other potential adverse effects including cytokine release syndromes, hypersensitivity reactions or autoimmune diseases.

Conclusion

Because of their structure, intended targets and the population to be treated, mAbs are medicinal products with a high immunotoxic potential, which is reflected in the clinical experience on mAbs-induced adverse effects accumulated over the recent years. Current non clinical immunotoxicity studies are inadequate to address all safety issues in relation to the possible immunotoxicity of mAbs, and clinical studies rarely include validated end-points dedicated to the prediction of immunotoxicity.

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