Are 6-months Repeat Dose Toxicity Studies in Nonhuman Primates for Monoclonal Antibodies really necessary?

Minor Research Project at Dutch Medicines Evaluation Board

Mohèb Elwakiel (4185730)

Supervisors: dr. Peter van Meer & dr. Jan Willem van der Laan

Are 6-months Repeat Dose Toxicity Studies in Nonhuman Primates for Monoclonal Antibodies really necessary?

Mohèb Elwakiel[1,2], Peter van Meer[1,2], Jan Willem van der Laan[2,*], Kathryn Chapman[3], Aukje Mantel-Teeuwisse[1] and Christine Gispen-de Wied[2]

1. Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences
2. Dutch Medicines Evaluation Board * jw.vd.laan@cbg-meb.nl
3. National Centre for the Replacement, Refinement and Reduction of Animals in Research

Why this study is of interest: Toxicity studies with a duration of up to 6 months are requested by health authorities to evaluate the safety of biopharmaceutics for chronic use. However, there is increasing evidence that adverse effects of monoclonal antibody-based therapies could be explained by either pharmacology or immune response.

Objective: In this project we investigated which proportion of adverse drug reactions can be detected in short-term repeat dose toxicity studies (≤ 1 month) and which new adverse drug reactions develop in long-term repeat dose toxicity studies (> 1 month) in nonhuman primates for monoclonal antibodies.

Results: Thirty new adverse drug reactions were identified in 11 of the 24 marketed monoclonal antibodies. As for the non-marketed monoclonal anitbodies, 12 new adverse drug reactions were found in 6 of the 24 products. The majority of the adverse drug reactions (marketed, 77%; non-marketed, 75%) can be explained by either pharmacology or immune response. The leading cause of toxicity was unknown for the remaining adverse drug reactions (marketed, 23%; non-marketed, 25%).

Conclusion: Short-term repeat dose toxicity studies (≤ 1 month) in nonhuman primates for monoclonal antibodies reveal similar risk profiles compared to long-term repeat dose toxicity studies (> 1 month). Newly identified adverse drug reactions did not influence the risk profiles of monoclonal antibodies. Repeat dose toxicity studies in nonhuman primates for monclonal antibodies can be reduced from 6 to 1 month.

Keywords: Monoclonal antibodies, nonhuman primates, repeat dose toxicity, duration, regulatory science, safety, International Conference on Harmonization S6 guideline

Introduction

Nonclinical development and regulations

Nonclinical studies need to be performed for every single new drug to investigate the mechanism of action, drug safety and efficacy and to determine a safe dose for in humans. A substantial and large component of the nonclinical program of the drug is animal testing. Every drug needs to be examined in animals, before the drug can be administered in man[1]. There are several nonclinical safety studies which need to be carried out in animals, such as safety pharmacology and pharmacodynamic studies, single- and repeat dose toxicity (RDT) studies, developmental- and reproductive toxicity studies, genotoxicity, carcinogenicity, immunogenicity and local tolerance studies[1]. Instructions and regulations can be found and are captured in the International Conference on Harmonization (ICH)-S6 guideline[1]. Data derived from animal studies need to be submitted and companies receive approval from health authorities before the drug can be tested in humans[1,2].

Key aspects to choose a relevant animal model are documented in the ICH-S6 guideline and literature[3]. The protection and welfare of animals is covered by the European Union (EU) legislation[3]. There are many rodent and non-rodent species available, such as rats, guinea pig, but also nonhuman primates (NHPs). The most relevant species need to be selected, based on the nature of the drugs and more specifically its pharmacological activity[1]. Animal testing is being performed based on the assumption that drug safety can be predicted in animals and extrapolated to humans[4,5]. However, animal testing has its drawbacks. It already has often happened that experiments in animals yielded very misleading results for humans. A well-known example is humanized monoclonal antibody (mAb) TGN1412 for treatment of β-cell chronic lymphocytic leukemia and rheumatoid arthritis[4]. This anti-CD28 mAb TGN1412 was tested in various animals, including NHPs and then on healthy human volunteers. In a phase 1 clinical trial, all 6 male subjects became seriously ill and they developed multiple organ deficiencies. The trial was stopped immediately. The drug was generally well tolerated in animals during a variety of toxicity studies. In humans, the drug caused severe inflammatory reactions[4].

Health authorities encourage researchers to investigate the possibilities to replace, refine and reduce animal testing with the purpose to work towards non-animal methods for nonclinical studies. Animal testing can give a wrong impression of how the drug works in humans. Animal testing does not guarantee that a drug is safe in humans. There are still no good alternatives for animal testing available. But there are options to reduce and refine animal experiments. Guidelines which concern nonclinical safety studies are ineffective.

Theoretical framework

RDT studies are mandatory according to the ICH-S6 guideline and necessary to identify and characterize all adverse drug reactions (ADRs) over time[1]. RDT studies mainly focus on target organs and tissue effects, but also examine dose-response

relationships and whether ADRs are reversible. For RDT studies, the dose regimen, duration of the study and route of administration should be in agreement with the intended clinical use[1,2]. The duration of RDT studies depend on clinical use of the drug, but studies up to 6 months are requested for biopharmaceutics for chronic use[1]. RDT studies up to 6 months are carried out to reveal the potential risks of a drug over a longer period of time. Biopharmaceutics prescribed for long-term use originally needed to be tested up to 9 months[6]. An evaluation of studies with a duration of 6 months and studies with longer duration suggested that RDT studies of 6 months were considered sufficient. RDT studies of 6 months revealed similar risk profiles compared to longer than 6 months RDT studies for 23 biotechnology-derived biopharmaceutics[6]. Newly identified ADRs in long-term RDT studies (> 6 months) were found for 2 drugs (Adalimumab and Insulin), but did not influence the drug safety profile.

Biotechnology-derived products for short-term use and life-threatening diseases are generally tested up to 2 weeks[2]. It is important to mention that RDT studies up to 6 months should be conducted in two relevant species, in one which must be a non-rodent[1,2]. Although in exceptional cases, according to the ICH-S6 guideline, only one relevant non-rodent species can be used when scientifically justified[1]. As an illustration, NHPs are often chosen as relevant animal model for mAbs, based on nature of the drugs and more specifically its pharmacological activity[7,8]. NHPs are a frequently used species for mAbs and the number of animals can be reduced by adapting the study design[9,10]. Chapman et al. investigated whether it was possible to reduce the use of NHPs in RDT studies for mAbs[10]. They suggested that the number of NHPs can be reduced from 144 to 52 primates and by performing 2 RDT studies (1 month and 1 further study) instead of 3 studies[10]. In another study was found that use of NHPs for mAbs is not always justified[9]. Thirty-three mAbs were approved on the market, in which 27 were tested in nonclinical studies which used NHPs as an animal model. The justification was not proper for 8 mAbs[9]. In addition, there is rapidly growing evidence on mAbs that ADRs induced by mAbs are predictable and can be either explained by pharmacology or immune response[,11,12 13].

Objectives and research question

The first objective in this project is to determine whether short-term RDT studies (≤ 1 month) in NHPs for mAbs reveal similar risk profiles compared to long-term RDT studies (> 1 month). The second objective is to determine how and whether newly identified ADRs in long-term RDT studies (> 1 month) affect the risk profile of the drug. To answer both objectives, we would like to investigate which proportion of ADRs can be detected in short-term RDT studies (≤ 1 month) and which new adverse reactions develop in long-term RDT studies (> 1 month) in NHPs for mAbs. If the conclusion that most adverse effects are (exaggerated) pharmacology, which can also be detected at early timepoints is true for all mAbs. The need for 6 months studies in addition to short-term RDT studies (≤ 1 month) is open for discussion. Would it be sufficient to conduct a short-term RDT study of 1 month to detect this exaggerated pharmacology?

Methods

MAbs which received a marketing authorization in the EU up to 01-10-2014 were identified from the European Medicines Agency (EMA) website and literature[14]. MAbs for which the safety was evaluated in RDT studies using NHPs as an animal model were identified via European Public Assessment Report (EPAR) and the drug registration file (Common Technical Document, CTD). The CTDs were accessed at the Dutch Medicines Evaluation Board (CBG-MEB). The CTD relating to [28] was not available. The nonclinical overview of [28] was obtained from the EMA through my daily supervisor.

The following variables were extracted from the RDT studies noted in the CTD: Study number and duration, animal model (relevance, race, gender, sample size), dose information (route of administration and dose levels) and ADRs induced by the mAbs. General characteristics and identifiers were gathered on mAbs: International nonproprietary name, brand name, indication, approval date, manufacturer, chronic vs. acute, type, isotype and target. The non-clinical overview, tabulated and written non-clinical summaries (module 2) and non-clinical study reports (module 4) were used to collect the information.

For the data-analysis, only mAbs with short-term (≤ 1 month) and long-term RDT studies (> 1 month) in NHPs were selected. Other mAbs were not taken in consideration, except [34] and [38]. The chosen mAbs were divided into three groups (3, 6 or more than 6 months), based on the longest RDT study.

ADRs identified in the short-term RDT (≤ 1 month) and long-term RDT studies (> 1 month) were compared. ADRs seen in short-term RDT (≤ 1 month) and long-term RDT studies (> 1 month) were eliminated, as well as the ADRs only found in the short-term RDT studies (≤ 1 month). Newly identified ADRs found in long-term RDT studies (> 1 month) were organized into 2 groups, based on the leading cause of toxicity (Pharmacology/immune response or unknown). Every single new ADR was discussed with my supervisors. Four steps were carried out to determine whether the new ADRs were related to pharmacology or immune response. 1). Are the new ADRs predictable from short-term RDT studies (≤ 1 month) 2). Nature of the ADRs (reversibel or not) and dose-related 3). What does the animal model and the mechanism of action, indication and drug target tell us 4). Information on newly identified ADRs explained by the manufacturer.

The Medical Dictionary for Regulatory Activities (MedDRA, developed by ICH, version 17.1) was used to categorize and anonymized the new ADRs found in long-term RDT studies (> 1 month). The available MedDRA manual with specific instructions was used to categorize the newly identified ADRs in the five hierarchy levels of MedDRA: Lowest Level Term (LLT), Preferred Term (PT), Highest Level Term (HLT), High Level Group Term (HLGT) and System Organ Class (SOC)[15].

To obtain short and long-term safety data on non-marketed products, a questionnaire was developed and distributed to pharmaceutical companies. The information was gathered through a questionnaire in collaboration with the National Centre for Refinement, Reduction and Replacement of Animals in Research. The survey can be found in the appendix 1.

Results

Short and long-term safety data on marketed mAbs

From the year 1988 to 2014, 41 mAbs were approved via the Marketing Authorization Application (Table 1)[14]. Six products are currently withdrawn from the market **[4, 5, 15, 18, 21, 41]**. **[18]** was the only drug removed from the market due to safety issues.

International Nonproprietary Name	NHPs used in RDT studies
1	Cynomolgus and Rhesus
2	Cynomolgus
3	Cynomolgus
4	None
5	None
6	Rhesus
7	Cynomolgus
8	Cynomolgus
9	Cynomolgus
10	Cynomolgus
11	Marmoset
12	None
13	Cynomolgus
14	Cynomolgus
15	Cynomolgus and Chimpanzees
16	Cynomolgus
17	None
18	Chimpanzees
19	Cynomolgus
20	Cynomolgus
21	None
22	Cynomolgus and Chimpanzees
23	Cynomolgus
24	None
25	Cynomolgus and Rhesus
26	Cynomolgus
27	Cynomolgus
28	Cynomolgus
29	None
30	Cynomolgus
31	Cynomolgus
32	Cynomolgus
33	Cynomolgus
34	Cynomolgus
35	None
36	Cynomolgus
37	Cynomolgus and Rhesus
38	Cynomolgus
39	Cynomolgus
40	Cynomolgus
41	Cynomolgus

Data is anonymized by removing any product names or codes or other indicators.

Thirty-three of the 41 products (80%) were tested in RDT studies which used NHPs as animal model. The predominately used NHP species was cynomolgus monkey. The products **[4, 5, 7, 21, 24, 35]** were not tested in NHPs. **[12, 29]** were tested in NHPs, but not in RDT studies. The drugs are prescribed for short-term use and no RDT studies were performed at all. For both drugs, the use of NHPs in other nonclinical studies was not justified.

Twenty-four of the 33 mAbs (73%) were used for the data-analysis (Fig. 1). Seven drugs for short-term use were eliminated, except **[31]**. For testing those type of mAbs, normally RDT studies up to 2 weeks are sufficient[2]. **[14, 22]**, both prescribed for long-term use were also discharged. The remaining 24 mAbs were divided into three groups. One product was tested up to 2 months. Therefore, it is the only mAb in group 1. Unlike, **[28]** which was categorized in the second group. **[30]** belongs to the same group, as well as **[18]** and **[40]**. The third and last group consisted of mAbs with short-term RDT studies (≤ 1 month) and longer than 6 months RDT studies. The majority (19 of the 24) of products were tested up to 6 months (79%). Justification for using NHPs was lacking, inadequate or not optimal for the products **[10, 11, 13, 25, 32]**) [9,14], displayed in appendix 2.

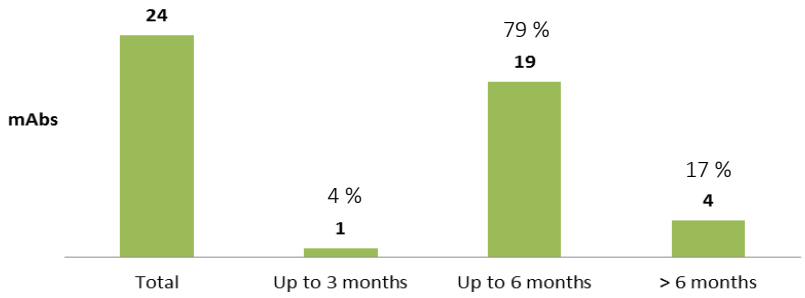

Figure 1. *Classification of mAbs based on the duration of the RTD studies.*

Thirty new ADRs were identified in long-term RDT studies (> 1 month) divided over 11 of the 24 mAbs. The leading cause of toxicity was unknown for 7 of the 30 ADRs (23%) (Fig. 2; Table 2). Noteworthy, those findings were for the first time seen and found between week 2 and 10 in the long-term RDT studies (> 1 month). Those ADRs were not predictable from short-term RDT studies (≤ 1 month) and were not related to the mechanism and target of the drug.

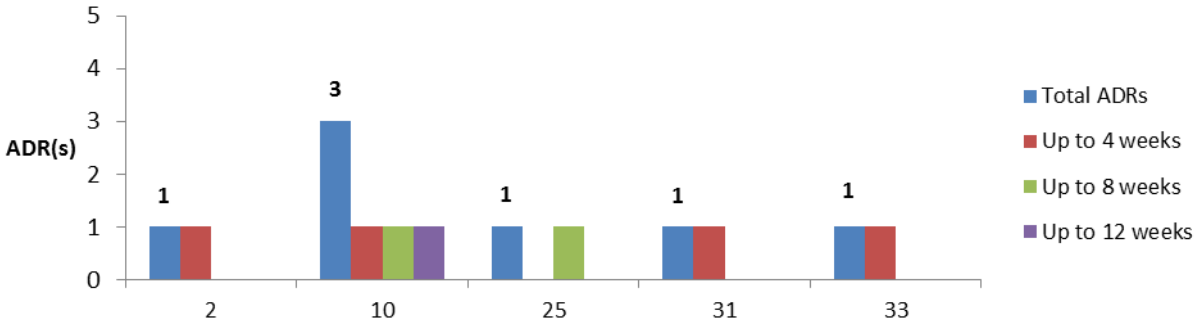

Figure 2. *The leading cause of toxicity was unknown for 7 of the 30 new ADRs in long-term RDT studies (> 1 month).*

The new ADRs for **[2]** and **[25]** were identified and explained in the literature[6]. All the 7 new ADRs are common and acceptable, based on the performed clinical studies and indication of the drugs. **[31]** and **[33]** are both anticancer drugs. The 3 new ADRs found for **[10]**. were reversibele and acceptable. The drug is also a anticancer drug **REMOVED INDICATION**. **REMOVED LARGE PART OF INFORMATION OBTAINED FROM THE CTD.**

Twenty-three of the 30 new ADRs (77%) in the long-term RDT studies (> 1 month) can be explained by either pharmacology or immune response (Fig. 3; Table 3). Most of those findings are seen for the first time at the end of the long-term RDT studies (> 1 month). It is however revealed that those ADRs are predictable (at an early stage) from the short-term RDT (≤ 1 month). Four steps were performed in order to determine whether those ADRs were related to pharmacology or immune response. The first step was to identify a trend (estimated course of a particular effect) or indicators in short-term RDT (≤ 1 month) which were related to the newly identified ADRs found in the long-term RDT studies (> 1 month).

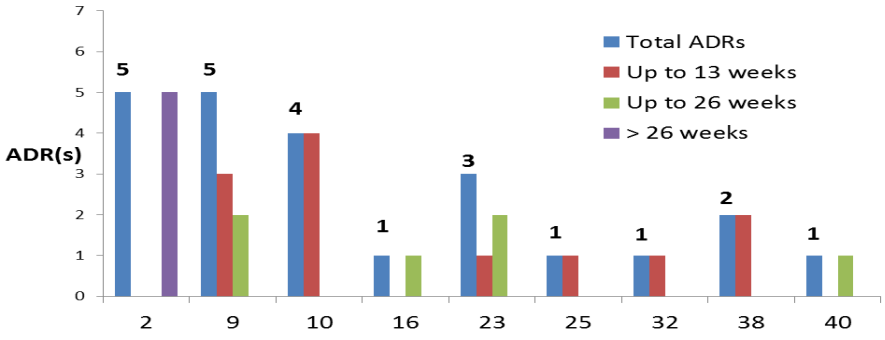

Figure 3. *Twenty-three of the 30 ADRs can be explained either by pharmacology or immunological reactions*.

For [2], new ADRs. In the short-term RDT studies (≤ 1 month), a slight change in thymus and spleen weight was observed (trend). But also other indicators were found, such as decreased cellularity of β-cells in splenic follicles, recruitement of neutrophils and monocytes to infection sites, the production of phagocytes. The second step was to identify whether the ADRs in the long-term RDT studies (> 1 month) were reversible and dose-related. In [2], both findings were reversible of nature. The findings were also dose-related and severity of the findings increased with mid and high dose levels. The reason why most of those ADRs were identified at the end of the long-term RDT studies (> 1 month) at sacrifice is simple. In the case of [2], those ADRs can be only detected in sacrificed animals through gross pathological changes and histopathological studies in tissue. In the short-term RDT (≤ 1 month) relevant indicators were found by performing pathological and histopathological studies in tissue. REMOVED SENTENCES OF CTD. The third step was to determine whether those ADRs were related to the mechanism and target of the drug. The indication of the drug was also considered, as well as the animal model. From the animal model interesting information can be extracted. Are the ADRs common in a specific animal model without medication? The answer was no on that question. [2], is a drug for the treatment of diseases REMOVED PART CTD. The last step was to look at information on the new ADRs provided by the company. This information was collected from the CTD. Every single new ADR was confirmed by the description from the CTD. REMOVED PART OF CTD. Based on the four steps, it could be said that the ADRs were predictable from short-term RDT studies (≤ 1 month) and were related to pharmacology or immune response. Not all new ADRs were the same, but all the steps were carried out each single time in all the mAbs. The most important factors in determine the leading cause of toxicity was by consulting the explanation of the manufacturer and by clarifying the correlation between the short-term RDT (≤ 1 month) and long-term RDT studies (> 1 month).

Table 2. *New ADRs for the first time identified in long-term RDT studies (> 1 month) were the leading cause of toxicity is unknown*

Products	Study Duration	MedDRA Coding Levels PT*	HLT	HLGT	SOC	Time point ADRs
[2]	REMOVED INDICATOR OF DRUG	Weight decreased [a]	Physical examination procedures & organ system status	Physical examination procedures & organ system topics	Investigations	Week 2
[10]	Up to 9 weeks	Haemosiderosis	Iron excess	Iron & trace metal metabolic disorders	Metabolic & nutrition disorders	Week 8
	9 weeks	Hypophosphatemia	Phosphorus metabolic disorders	Bone, calcium, magnesium & phospurus metabolic disorders	Metabolic & nutrition disorders	Week 10
	24 weeks	Malaise [b]	Asthenic disorders	General system disorders	General disorders & administration site conditions	Week 2
		Decreased activity [c]				
		Asthenia [d]				
[25]	24 weeks	Somnolence [e]	Disturbances in consciousness	Neurological disorders	Nervous system disorders	Week 7
		Mucosal discoloration [f]	Mucosal findings abnormal	General system disorders	General disorders & administration site conditions	
		Heart rate increased	Heart rate & pulse investigations	Cardiac & vascular investigations (excl. enzyme test)	Investigations	
[31]	7 weeks	Diarrhea	Diarrhea (excl. infective)	Gastrointestinal motility & defecation conditions	Gastrointestinal disorders	Week 1
[33]	8 weeks	Vomiting	Nausea & vomiting symptoms	Gastrointestinal signs & symptoms	Gastrointestinal disorders	Week 1

* In most cases the PT corresponds to the LLT. Exceptions: a = Weight loss; b = Generally off-color; c = Hypoactive; d = Debility; e = Drowsiness; f = Pale mucous membrane. One of the findings of [10] and the finding of [25] were explained by multiple MedDRA terms.

Products	Study Duration	Correlation ADRs in short-term RDT study (≤ 1 month)	PT*	MedDRA Coding Levels HLT	SOC	Time point ADRs
[2]	REMOVED FROM REPORT	Change thymus weight (trend)	Thymus hypoplasia	Thymus disorder	Blood & lymphatic system disorders	End of study
		Change spleen weight (trend)	Splenic rupture	Spleen disorders	Blood & lymphatic system disorders	End of study
		Change spleen weight (trend)	Splenic rupture	Spleen disorders	Blood & lymphatic system disorders	End of study
		Change number lymphocytes (trend)	Decrease Natural killer cells [a]	White blood cell analysis	Investigations	REMOVED
		Change number lymphocytes (trend)	Decrease B-lymphocytes [b]	White blood cell analysis	Investigations	REMOVED
[9]	13 weeks	No trend was visible, decision based on literature[14,21].	Ovarian mass	Ovarian & fallopian tube disorders	Reproductive system & breast disorders	End of study
			Uterus rupture	Uterus disorders		
	13 weeks	No trend was visible, decision based on literature[14,21].	Anovulation cycle [c]	Menstruation & uterine bleeding	Reproductive system & breast disorders	End of study
	13 weeks	Dysplasia physeal, degeneration of the cartilage matrix, bone marrow	Epiphyseal injury [d]	Epiphyseal disorders	Musculoskeletal & connective tissue disorders	End of study
	26 weeks	Anovulation cycle	Anovulation cycle [e]	Menstruation & uterine bleeding	Reproductive system & breast disorders	End of study
	26 weeks	Ovarian mass and uterus rupture	Not specified [f]	Ovarian/fallopian tube disorders	Reproductive system & breast disorders	End of study
[10]	Up to 9 weeks	Decrease in number lymphocytes	Granolocytes	White blood cell analysis	Investigations	End of study
			Polycythaemia [g]	Polycythaemia	Blood & lymphatic system disorders	
	9 weeks	Mild bone marrow alterations	Not specified [h]	Bone marrow & immune tissue histopathology procedures	Investigations	End of study
	9 weeks	Decrease in number lymphocytes	Thymus hypoplasia Lymphopenia	Thymus disorder Leukopenias	Blood & lymphatic system disorders	End of study
	24 weeks	Decrease in number lymphocytes	White blood cell count abnormal [i]	White blood cell analysis	Investigations	Week 6
[16]	24-48 weeks	Increase total and cortical bone mineral density, other bone markers	Bone formation test	Biochemical markers of bone metabolism	Investigations	Week 24
[23]	12 weeks	Diarrhea, dose-related inflammation in gastrointestinal tract	Enterocolitis	Gastrointestinal inflammatory disorders	Gastrointestinal disorders	Week 6
	16 weeks	Rash and/or reddening of the skin	Dermatitis	Dermatitis and eczema	Skin and subcutaneous tissue disorders	Week 17
	24 weeks	Changes in thymus and spleen	Endocrine test	Endocrine analyze and imaging	Investigations	Week 23
[25]	24 weeks	Immune complex deposition, other antibody-dependent phenomena	Glomerulonephritis	Glomerulonephritis & nephrotic syndrome	Renal & urinary disorders	Week 12 #
[32]	13 weeks	Inflammation, development of new blood vessels	Angiogram abnormal [j]	Vascular imaging procedures	Investigations	End of study
[38]	12 weeks	Lymphoid depletion in the spleen and thymus	Thymus hypoplasia	Immune system abnormalities congenital	Congenital familial & genetic disorders	Week 9
	12 weeks	Hematologic/bone marrow toxicity	Hepatobiliary signs & symptoms [k]	Liver & spleen enlargement	Blood & lymphatic disorders	Week 9
[40]	26 weeks	Lymphoid hyperplasia	Lymphadenopathy [l]	Lymphatic system disorders	Blood & lymphatic system disorders	End of study

Table 3. *New ADRs for the first time identified in long-term RDT studies (> 1 month) which were attributed to pharmacolgical attributes of the drug or immune response.*

* In most cases the PT corresponds to the LLT. Exceptions: a = CD56 lymphocytes decreased; b = CD20 lymphocytes decreased; c = Anovulation; d = Growth plate injury; e = Anovulation; f = LLT/PT could not be determined; g = Erythrocytosis; h = LLT/PT could not be determined; i = LLT could not be determined; j = Fluorescein angiography abnormal; k = Hepatosplenomegaly; l = Lymphoid nodule. One of the findings of **[9]** and 2 findings of **[10]** were explained by multiple MedDRA terms. # Leading cause of toxicity is for all findings exaggerated pharmacology, except for **[25]**, immune response.

Short and long-term safety data on non-marketed products

Companies provided information on 24 mAbs of which 18 were tested in NHPs and 6 in NHPs in combination with rodent species. The data was given from 2 firms, 1 of medium size (FTEs: 10.000 to 25.000) and 1 multinational (FTEs: 25.000 to 100.000).

Twelve new ADRs were found in 6 of the 24 products (Table 4). Nine of the 12 new ADRs (75%) in long-term RDT studies (> 1 month) can be explained by the pharmacological properties of the drug or immunological reactions. The leading cause of toxicity was unknown for 3 new adverse drug reactions (25%). Those ADRs were detected up to 3 months.

The 6 mAbs proceeded to clinical trials. Development of 3 products was halted due to lack of efficacy. Two mAbs are still in clinical development. One of the products did not appear on the market, because it was business decision to discharge this drug from the clinical trials. It was not due to a particular efficacy or safety concern.

Table 4. *New ADRs in long-term RDT studies (> 1 month) for products which have not appeared on market or still in development.*

Product	**Timepoint ADRs**	**Toxicity finding(s)**	**Leading cause of toxicity**	**Terminated at Clinical Studies**
1	9 months	Skin, urogenital and gastrointestinal tract findings	Intended target	Lack of efficacy
2	9 months	Severe injection site reaction in a single animal	Immune response	Not applicable
3	9 months	Regenerative anemia in a single animal	Immune response	Business decision
4	6 months	Skin related findings	Intended target	Not applicable
5*	3 month	Anemia (decreased red cell mass) Change in lymphoid organs & spleen weight	Unknown	Lack of efficacy
6	3 months	Increased heart weight hypertrophy (heart, bile duct, interstitial endothelium) Proteinuria and anemia (decreased red cell mass) Hepatic portal inflammation/fibrosis.	Intended target	Lack of efficacy

* Additional information from the manufacturer: The 2 new ADRs were seen at 3 and 9 months with med and high dose. It is uncertain if these changes are target related, however the target is widely expressed in those tissues and this was confirmed in a tissue cross reactivity study. Additionally, those 2 ADRs are often seen with other non-marketed mAbs in short-term RDT studies (≤ 1 month) and of no real safety concern.

Discussion

General Discussion

There were 41 mAbs identified from the EMA website. Thirty-three products (80%) were tested in RDT which used NHPs as animal model. Twenty-four developmental programs (73%) included short-term (≤ 1 month) and long-term RDT studies (> 1 month). Thirty new ADRs were identified in 11 of the 24 mAbs which were approved on the market from 1998 to 2014. As for the non-marketed mAbs, 12 new ADRs were found in 6 of 24 products. The majority of those new ADRs (marketed mAbs, 77%; non-marketed mAbs, 75%) can be explained either by pharmacology or immune response. This study confirmed and corresponds to previous research that most of the new ADRs induced by mAbs are highly predictable and mediated by either pharmacology or immune response[12,13]. The leading cause of toxicity was unknown for the remaining new ADRS (marketed mAbs, 23%; non-marketed mAbs, 25%). Occasionally newly identified ADRs are found without knowing the origin[4,6,11]. The data derived from the survey confirmed and strengthened the results of the marketed authorized mAbs.

The first and second objective from this research project can be confirmed. Most of the drugs (marketed mAbs, 54%; non-marketed mAbs, 75%) revealed that short-term RDT studies (≤ 1 month) and long-term RDT studies (> 1 month) in NHPs for mAbs have identical drug safety profiles. In addition, the newly identified ADRs in the long-term RDT studies (> 1 month) were of no safety concern. The results from our study are in large extent consistent and comparable with the study of Clarke et al., which made it possible to change the ICH-S6 guideline with regard to duration of RDT studies[6]. The duration of the RDT studies was changed from 9 to 6 months. The reason for changing the ICH-S6 guideline was due to the fact that studies up to 6 months showed similar drug risk profiles compared to longer duration studies for 23 biopharmaceutics[6]. In our case, the newly identified ADRs did not contribute to the drug safety profiles. The majority of the new ADRs are predictable, related to pharmacology or immune response and could be detected via short-term RDT (≤ 1 month). The leading cause of toxicity was unknown for the remaining ADRs. Those ADRs were not predictable and were not related to mechanism and target of the drug. But if those findings had resulted in safety concerns. RDT studies could be reduced to 3 months, because all those new ADRs were found up to 3 months including the results from the survey. Yet those ADRs are of no significance. Repeat dose toxicity studies in NHPs for mAbs can be reduced from 6 to 1 month. Additional studies are not needed. As of now, data derived from this research project need to be translated into the ICH-S6 guideline, in order to evaluate the safety of mAbs in RDT studies of 1 month. And by using NHPs as an animal model, if it is scientifically justified[1,7,8].

Theoretical implications and future directions

As described above, it is now clear what kind of new ADRs induced by mAbs can be expected or could be found in short-term and long-term RDT studies which used NHPs as an animal model. The newly identified ADRs were mostly predictable and related to

pharmacology or immune response. The ADRs were the leading cause of toxicity is unknown were acceptable due to the indication and nature of the drugs.

For future research it would be an asset to this project to collect more information on non-marketed mAbs. Two companies have send information on mAbs, in total 24 drugs. Those 24 mAbs are just a small percentage of the drugs in the development phase (preclinical and clinical trials). However, the data is of course useful and the number of products provided clear evidence to confirm the data from the marketed mAbs. In addition, it would be helpful if the industry provides more detailed information on the new ADRs in long-term RDT studies (> 1 month). Correlations with short-term RDT studies (≤ 1 month), the exact time point of the ADRs and more information on the mechanism and target of the mAbs. But also the reason why NHPs were chosen as a relevant animal model. This ensures that non-marketed and marketed products can be treated as one. The four steps to determine whether the ADRs are pharmacology or immune response and its predictability could than be analysed for the non-marketed products. The data from the industry now needed to be taken for granted.

Another caveat was that the justification for using NHPs was lacking, inadequate or not optimal for the products **[13, 10, 11, 25, 32]**)[9,14], displayed in appendix 2. However, those marketed mAbs were taken in consideration for the data-analysis. Those products were included in the data-analysis, to obtain as much as possible, data on developmental programs which included short-term (≤ 1 month) and long-term RDT studies (> 1 month). For all other products which were included in the data-analysis, NHPs were the pharmacologically relevant species[9,14]. Last but not least, 24 of the 41 mAbs were used in the data-analysis. The remaining 17 drugs were accessible. Assessing those CTDs was time consuming and not relevant for this project, developmental programs only included short-term RDT studies (≤ 1 month) or did not use NHPs as animal model.

Conclusion

Short-term RDT studies (≤ 1 month) in NHPs for mAbs reveal similar risk profiles compared to long-term RDT studies (> 1 month). In only 11 of the 24 marketed mAbs (46%) new ADRs were identified in long-term RDT studies (> 1 month). As for the non-marketed mAbs, 6 of the 24 products (25%) showed new ADRs in long-term RDT studies (> 1 month). For marketed mAbs (77%) and non-marketed mAbs (75%), the majority of the ADRs can be explained by either pharmacology or immune respons. The leading cause of toxicity was unknown for the remaining ADRs (marketed mAbs, 23%; non-marketed mAbs, 25%). The newly identified ADRs in the long-term RDT studies (> 1 month) were of no safety concern. On the basis of the evidence currently available, we recommend that studies up to 6 months in NHPs for mAbs can be reduced to 1 month RDT studies, resulting in a change in the ICH-S6 guideline.

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Abbreviations

ADR	Adverse Drug Reaction (ADRs plural.)
CBG-MEB	Dutch Medicines Evaluation Board
CTD	Common Technical Document (CTDs plural.)
EMA	European Medicines Agency
EPAR	European Public Assessment Report (EPARs plur.)
EU	European Union
HLGT	High Level Group Term (HLGTs plur.)
HLT	High Level Term (HLTs plur.)
ICH	International Conference on Harmonization
LLT	Low Level Term (LLTs plur.)
MedDRA	Medical Dictionary for Regulatory Activities
mAb	Monoclonal antibody (mAbs plur.)
NHP	Nonhuman primate (NHPs plur.)
PT	Preferred Term (PTs plur.)
RDT	Repeat Dose Toxicity
SOC	System Organ Class (SOCs plur.)

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Appendix 1

Dutch Medicines Evaluation Board Survey

Toxicity studies with a duration of up to 6 months are requested by health authorities to evaluate the safety of new biotechnology-derived products for chronic use[1]. An evaluation of studies with a duration of 6 months and studies with a longer duration suggested that repeat dose toxicity studies of 6 months in non-rodents are considered sufficient[2]. In addition, there is increasing evidence that adverse effects of monoclonal antibody-based therapies (mAbs) could be explained by either pharmacology or immune response[3]. In this survey, we would like to investigate which proportion of adverse reactions can be detected in short term studies (up to 1 month) and which new adverse reactions develop in 6 month studies for products that are currently in development or were withdrawn. If the conclusion that most adverse effects are (exaggerated) pharmacology, which can also be detected at early timepoints is true for all mAbs, including not yet registered products the need for 6 months studies in addition to short term studies is open for discussion. Would it be sufficient to conduct a 1 month study to detect this exaggerated pharmacology?

The Dutch Medicines Evaluation Board, in collaboration with Utrecht University, will compare the outcomes of 1 vs 6 months nonclinical studies for mAbs. Because the Medicines Evaluation Board only has access to market authorized mAbs, we are interested in collecting data from unpublished compounds that may have been dropped from development because of adverse effects observed in pharmacologically relevant species. We are also interested in molecules which are not terminated but are still in development and not yet in the public domain.

Therefore, we request your assistance and invite you to fill in this questionnaire, which will enable us to combine information from the pharmaceutical industry and regulatory authorities.

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Contact information:

Dr. Peter van Meer, Assessor, Medicines Evaluation Board

E-mail: p.v.meer@cbg-meb.nl, Phone: +31 88 224 84 68

Data analysis and public disclosure:

It is the intention of the researchers to publish the results of their research in a peer-reviewed journal. Data will be handled in a confidential manner. Data will be appropriately anonymized by removing any product names or codes. Data is analyzed at an aggregated level to prevent product identification unless explicit permission is given by the company to discuss individual cases. Only aggregated data and pre-approved individual cases will be presented for publication. No company-specific data or estimates will be reported, except with permission of the company. Data will not be shared with other parties or used for research other than that which is described here without permission of the company.

Instructions:

Please fill in the answers to the each question, without changing its format. The survey consists of three sections: a general section, a case by case section and a final section. In the case by case section, please use the original template for each new product/case. There may be questions which are not applicable for each product. Please check the NA (not applicable) box or enter "NA" in the text-box. Upon completing the survey, please save your document(s) as PDF and send it as an email attachment to p.v.meer@cbg-meb.nl It would be appreciated if the questionnaire could be completed and submitted no later than 15 February 2014.

Section 1: General Questions

1) What is the size of your company in FTEs?

☐ 100.000 and above

☐ 25.000 to 100.000

☐ 10.000 to 25.000

☐ 1.000 to 10.000

☐ Less than 1.000

2) How many unregistered mAbs have progressed from short-term studies (up to 1 month) to longer duration studies (up to 6 months)?

3) How many programs were conducted in NHP only, rodent only, NHP and rodent, other non-rodent, other non-rodent and rodent?

☐ NHP:

☐ Rodent:

☐ NHP and rodent:

☐ Other non-rodent species:

☐ Other non-rodent species and rodent:

4) How many had a new toxicity or a new immune-related finding in a longer duration study up to 6 months that was not observed in a shorter study?

5) How many of these were seen in studies with NHP only, rodent only, NHP and rodent, other non-rodent, other non-rodent and rodent?

☐ NHP:

☐ Rodent:

☐ NHP and rodent:

☐ Other non-rodent species:

☐ Other non-rodent species and rodent:

Proceed to section 2

Section 2: Case by case questions

Case by case reports are needed to gain in-depth information on question 4. If required, please use a new form (Section 2: Case by case questions) for every new case. Report cases in the textbox labeled casenumber as: Product N: ABC-00N, where ABC is your distinct manufacturer abbreviation consisting of three letters and N is a sequential number, starting with 001. The manufacturer abbreviation will be replaced with a non-identifying code to anonymize the data. When discussing findings, it may be relevant to discuss the target or pharmacology of the product, which may be named as a development code or INN. Both

development code and INN will be replaced with anonymized identifiers. Product specific data will not be disclosed without explicit approval of the company. The anonymized data is aggregated for further analysis.

Example Company name: **M**edicines **E**valuation **B**oard Case: 1 Casenumber: MEB-001

Case:

6a) Which intended toxicity duration studies were performed (please tick all that apply)?

☐ 1-month study

☐ 3-months study

☐ 6-months study

☐ Other, namely:

6b) If due to specific toxicity, in which study was the new finding observed (please tick all that apply)?

☐ In the 1-month study

☐ In the 3-months study

☐ In the 6-months study

☐ Other, namely:

- *If 'multiple new findings were observed', please fill in question 6c and 6d.*

6c) Please describe the multiple findings and provide their duration(s)?

☐ Not applicable

6d) If the product was terminated, which finding was considered to be the most important?

☐ Not applicable

6e) What did you consider to be leading cause of this new toxicity finding?

☐ Intended target

☐ Unintended target

☐ Immune response (incl. immunogenicity)

☐ Unknown

6f) If due to immune response, in which study was the new finding observed (please tick all that apply)?

☐ In the 1-month study

☐ In the 3-months study

☐ In the 6-months study

☐ Other, namely:

6g) If the drug development program was cancelled during clinical trials, was this due to the observed phenomenon:

☐ Lack of efficacy

☐ Safety issues Specify ☐ Nonclinical safety ☐ Clinical safety

☐ Not disclosed

- *If you answered 'safety issues with non clinical safety issues', please also fill in question 6h.*

6h) Please provide a brief answer explaining your response

☐ Not applicable

For a new case, use the provided Section 2 template (case by case questions) or otherwise, proceed to Section 3.

Section 3: Final Questions

7) Do you have any remarks about any of your reported case studies?

8) Do you have any remarks and/or suggestions related to this survey?

9) It would be extremely informative for us to have additional study data. Would you be willing to share additional information with us under a confidentiality agreement?

☐ Yes

☐ No

- *If 'yes', please fill in the contact information in question 10.*

10) Contact information of your contact person in the firm:

Name: Telephone: E-mail:

☐ Not applicable

This is the end of the survey. Thank you for your assistance in completing the survey!

Appendix 2

Justification of the use of NHPs as relevant model

Table 5. *Justification for using NHP was lacking, inadequate or not optimal.* Removed source

Product	Justification for NHP use
[3] *, [13]	Reduced affinity and specificity in NHP
[10], [29]*	Commonly used species for biotech development
[11], [12]*	NHP species were not relevant model
[25]	More non-rodent species are available as suitable model
[32]	Almost identical sequence of target (but without data on affinity)
[40]*	No explanation/justification given

* The marketed mAbs were not included in the data-analysis of this project because developmental programs consisted short-term RDT studies (≤ 1 month).