Safety Pharmacology Evaluation of Biopharmaceuticals

Article *in* Handbook of experimental pharmacology · June 2015

DOI: 10.1007/978-3-662-46943-9_15

CITATIONS
3

READS
478

3 authors:

Hamid Amouzadeh
Amgen
28 PUBLICATIONS **949** CITATIONS
SEE PROFILE

Michael J Engwall
Amgen
19 PUBLICATIONS **383** CITATIONS
SEE PROFILE

Hugo M Vargas
Amgen
146 PUBLICATIONS **2,206** CITATIONS
SEE PROFILE

Safety Pharmacology Evaluation of Biopharmaceuticals

Hamid R. Amouzadeh, Michael J. Engwall, and Hugo M. Vargas

Contents

Abstract

Biotechnology-derived pharmaceuticals or biopharmaceuticals (BPs) are molecules such as monoclonal antibodies, soluble/decoy receptors, hormones, enzymes, cytokines, and growth factors that are produced in various biological expression systems and are used to diagnose, treat, or prevent various diseases. Safety pharmacology (SP) assessment of BPs has evolved since the approval of the first BP (recombinant human insulin) in 1982. This evolution is ongoing and

H.R. Amouzadeh
Global Patient Safety, Global Regulatory Affairs & Safety, Amgen Inc, Thousand Oaks, CA, USA

M.J. Engwall • H.M. Vargas (✉)
Integrated Discovery & Safety Pharmacology, Amgen Inc, Thousand Oaks, CA, USA
e-mail: hvargas@amgen.com

M.K. Pugsley, M.J. Curtis (eds.), *Principles of Safety Pharmacology*, Handbook of Experimental Pharmacology 229, DOI 10.1007/978-3-662-46943-9_15

is informed by various international harmonization guidelines. Based on these guidelines, the potential undesirable effect of every drug candidate (small molecule or BP) on the cardiovascular, central nervous, and respiratory systems, referred to as the "core battery," should be assessed prior to first-in-human administration. However, SP assessment of BPs poses unique challenges such as choice of test species and integration of SP parameters into repeat-dose toxicity studies. This chapter reviews the evolution of SP assessment of BPs using the approval packages of marketed BPs and discusses the past, current, and new and upcoming approach and methods that can be used to generate high-quality data for the assessment of SP of BPs.

Keywords

Biopharmaceuticals • Cardiovascular system • Central nervous system • International Conference on Harmonization • Respiratory system • Safety pharmacology

1 Introduction

Biotechnology-derived pharmaceuticals or biopharmaceuticals (BPs) are molecules such as monoclonal antibodies (mAb), soluble/decoy receptors, hormones, enzymes, cytokines, and growth factors that are produced in various biological expression systems and are used to diagnose, treat, or prevent various diseases (International Conference on Harmonization (ICH) S6(R1) 2011). Other types of novel therapeutics, such as vaccines and oligonucleotides synthesized by bacterial or mammalian cells, are also considered BPs according to the ICH S6 (R1) guideline.

The features of BPs that distinguish them from traditional small molecule (SM) therapeutics are their relative larger physical size and molecular weight (typically $<$500 Da for SMs and $>$1,000 Da for BPs), molecular complexity, and unique selectivity for the intended therapeutic target. Because of this latter characteristic, BPs are expected to have less off-target activities relative to SM therapeutics and consequently have a reduced risk of off-target adverse effects in humans (Giezen et al. 2008; Amouzadeh and Vargas 2013). Important properties of BPs and SM therapeutics are listed in Table 1.

Innovative variations in the engineering of BPs have been developed in an effort to create new therapeutics to treat human disease. For example, peptibodies, which are peptides fused to an IgG Fc molecule, have emerged. The only marketed peptibody, romiplostim (NPLATE®), was approved under a Biologic License Application (BLA) by the United States Food and Drug Administration (FDA) in 2008 and by European Medicines Agency (EMA) in 2009 for the treatment of immune thrombocytopenic purpura (Shimamoto et al. 2012).

Table 1 Comparison of general characteristics of small molecules and biopharmaceuticals

Attribute	Small molecules	Biopharmaceuticals
Modality	Synthetic chemicals	mAb, peptides, peptibodies, fusion proteins, ADC, BiTEs®, and vaccines
Synthesis	Chemical	Biotechnological
Physiochemical properties	Well-defined, single molecule	Complex, heterogeneous
Molecular mass	<500–1,000 Da	>1,000 Da
Stability	Stable	Sensitive to heat and shear
Target selectivity	Low to high	High
Typical route	Oral	Parenteral
Distribution	Widespread	Plasma and extracellular space
Metabolism	• Inactive and active metabolites • CYP inhibition/induction • Covalent binding	Amino acids
Half-life	Short (<24 h)	Long (days to weeks)
Disposition	Linear or nonlinear PK	PK: altered by ADA and TMDD
Bioanalytical methods	LC/MS	Bioassay
Drug–drug interaction	High—both PK and PD	Low—mostly PD
Immunogenicity	Rare	Possible
Regulatory guidelines	ICH M3, S7A, and S7B	ICH S6R1, S7A and B
Typical species of choice	Rodent and non-rodent	NHP
Safety pharmacology Core battery	Dedicated	Integrated into toxicity studies
QT liability	• hERG assay • QT_c assay (non-rodent)	QT_c assay (non-rodent)
Toxicity	"On-/off-target"	"On-target" (exaggerated pharmacology)

mAb monoclonal antibody, *ADC* antibody-drug conjugates, *BiTEs* bispecific T cell-engaging antibodies, *Da* dalton, *CYP* cytochrome P-450, *PK* pharmacokinetic, *ADA* anti-drug antibody, *TMDD* target-mediated drug disposition, *LC/MS* liquid chromatography/mass spectroscopy, *PD* pharmacodynamic, *ICH* international conference on harmonization, *NHPs* nonhuman primates

Other examples of modified BPs are the antibody-drug conjugates (ADCs), which are used primarily as oncology therapeutics. These particular molecules are mAb-SM drug hybrids that are designed to take advantage of the selectivity of mAbs to deliver small cytotoxic molecules to specific tumor cells (Perez et al. 2014). Three ADCs have been approved by FDA thus far. These include gemtuzumab ozogamicin (MYLOTARG®) which targets CD33 for acute myelogenous leukemia, brentuximab vedotin (ADCETRIS®) which targets CD30 for Hodgkin lymphoma and anaplastic large cell lymphoma, and ado-trastuzumab emtansine (KADCYLA®) which targets HER2 positive metastatic breast cancer (Drugs@FDA). Although all of these molecules are classified as ADCs, it is

noteworthy that the first was reviewed by the FDA under a New Drug Application (NDA) as an SM, and the latter two were reviewed as BLAs (Drugs@FDA).

A more recent class of novel BPs in clinical development is bispecific T cell-engaging antibodies (BiTE®). Bispecific antibodies, in general, are engineered to recognize two distinct epitopes. BiTE® antibodies are comprised of two flexibly linked single-chain variable fragments of different antibodies, one directed against a tumor antigen and one targeting CD3 on T cells. As a result, these bispecific antibodies can transiently link tumor cells with resting polyclonal T cells to induce a surface target antigen-dependent redirected lysis of tumor cells. This pharmacological action closely mimics the natural cytotoxic T cell response and leads to the selective destruction of cancer cells. Blinatumomab (targets CD19 antigen on B cells) and solitomab (targets the epithelial cell adhesion molecule (EpCAM) antigen) are examples of BiTE®s for treatment of blood, lung, and gastrointestinal (GI) cancers, respectively (Frankel and Baeuerle 2013). The first BiTE® antibody, blinatumumab (BLINCYTO) BLINCYTO®, was approved in 2014 under accelerated approval program for the treatment of Philadelphia chromosome-negative relapsed or refractory B cell precursor acute lymphoblastic leukemia (Drugs@FDA).

The first BP, recombinant insulin, was approved in 1982 (Marafino and Pugsley 2003). Since then, there has been an increasing number of BPs approved for various indications. By the end of 2014, a total of 111 novel BPs have been approved by the FDA (Drugs@FDA). A survey of new drug approval trends (1994–2004) indicated that BPs have a better chance of attaining regulatory approval than conventional SM therapeutics (32 % vs. 12 %, respectively; DiMasi et al. 2010). This is due to low success rate in developing SMs for discrete targets such as the central nervous system (CNS), which has an 8 % success rate and a lower rate of attrition among BPs (DiMasi et al. 2010; Giezen et al. 2008). The latter is supported by recent data showing a withdrawal rate of 5 % for BPs and 9 % for SMs during 1998–2008 (DiMasi et al. 2010). Three BPs, efalizumab (RAPTIVA®; FDA 2009), gemtuzumab ozogamicin (MYLOTARG®; FDA 2010a), and peginesatide (OMONTYS®; FDA 2013a) were withdrawn from human use because of increased risk of progressive multifocal leukoencephalopathy (a rare and usually fatal disease caused by activation of the JC virus by a combination of pharmacological agents and immune compromise), lack of efficacy and safety concerns, and serious hypersensitivity reactions, respectively.

2 Safety Pharmacology Evaluation of Biopharmaceuticals: A Changing Landscape

Safety pharmacology (SP) assessment of BPs has evolved since the approval of recombinant human insulin in 1982. A recent review of the FDA approval packages for BLAs demonstrated diverse approaches towards SP assessment of BPs. Among the 111 BPs that have been approved (1980–2014: BLA: 110; NDA: 2), a variety of SP assessment strategies have been used. Among these BLA packages, 27 had no

specific pharmacology/toxicology reviews, 32 indicated that no SP studies were performed, 21 had SP evaluation integrated into toxicity studies, and 31 had dedicated SP studies. Overall, 62 % of the BLAs with a reported nonclinical safety summary cited SP information collected from dedicated or integrated studies (Table 2). However, many of the integrated and dedicated SP studies did not include all the required cardiovascular, CNS, respiratory assessments, i.e., core battery (Table 2). An important conclusion from this retrospective review is that various SP approaches have been applied in the evaluation of approved BPs. This is perhaps due to the fact that regulatory guidelines are intentionally not prescriptive, to allows sponsors discretion in the nonclinical safety assessment strategy used for their unique drug candidate and to accommodate innovative methods and approaches such as telemetry and integrated study design, e.g., the capture of SP functional endpoints in toxicity studies.

The evolution of SP, and toxicology practices for BPs, is ongoing and will be influenced by many factors, including (1) the novel scientific attributes and liabilities of new BPs associated with their mechanism of action and molecular targets, (2) the emergence of new methods or technologies to improve SP assessment, and (3) the ability to integrate "fit for purpose" functional evaluations in repeat-dose toxicity studies. The opportunities to introduce quality SP assessments into toxicity studies, as well as the pitfalls to consider, are highlighted in an excellent review article by Redfern et al. (2013). A key limitation of typical SP studies is that they are designed primarily as acute (single-dose) experiments, so functional effects that intensify or diminish (due to tolerance) with longer exposure are not evaluated systematically. This limitation represents a gap in the ability to perform clinical risk assessments based on functional hazards that occur with chronic dosing. This safety assessment gap can be mitigated by introducing sensitive SP evaluations into repeat-dose toxicity studies or performing dedicated repeat-dose SP studies. The changing landscape of SP evaluation for BPs and SMs is underscored by a recent pharmaceutical industry survey, which reported that many

Table 2 A survey of safety pharmacology information for novel BPs approved by US-FDA from 1980 to 2014

Modality	BLA[a]	Dedicated		Integrated		None	No data
		Core	Partial	Core	Partial		
Antibodies	41	2	6	4	11	9	10
Proteins/peptides	30	7	7	1	3	9	4
Enzymes	20	4	4	2	0	8	6
Cytokines	16	2	1	0	0	6	7
ADC	3	1	2	0	0	0	0
BiTE®	1	1	1	0	0	0	0
Total	111	17	14	7	14	32	27

Dedicated: Specific cardiovascular, neurobehavioral, and respiratory studies conducted
Integrated: SP endpoints were collected in toxicology studies
Core: all three assays; partial: only one or two assays; ADC: antibody-drug conjugate
[a]2 BP were approved as NDA: pasireotide (peptide) and gemtuzumab ozogamicin (ADC)

drug sponsors are actively using improved functional methods, like jacket-based telemetry systems for noninvasive cardiovascular (CV) monitoring, to detect functional effects after acute and chronic treatment in exploratory or Investigational New Drug (IND)-enabling toxicity studies (Authier et al. 2013).

3 Regulatory Guidelines

The overall goal of guidelines addressing the SP profiling of new therapeutics is to assure human safety upon first administration of novel drug candidates, and beyond. These guidelines include the general approaches on the nonclinical safety evaluation of drug candidates. Because of the unique nature of each drug candidate, either SM or BP, its safety assessment should be based on sound scientific rationale. This will allow for adequate characterization of the safety profile of a drug candidate prior to first administration to humans.

Safety pharmacology assessment of BPs is based on ICH M3(R2) (2009), S6 (R1) (2011), S7A (2000), and S7B (2005) guidelines. With regard to BPs, the M3 (R2) guideline addresses only the "timing of nonclinical studies relative to clinical development" and defers to S6 guideline for nonclinical safety assessment of biotechnology-derived drugs. The S6(R1) guideline indicates that "It is important to investigate the potential for undesirable pharmacological activity in appropriate animal models and, where necessary, to incorporate particular monitoring for these activities in the toxicity studies and/or clinical studies" (Section 4.1, ICH S6 (R1) 2011). Safety pharmacology assessment of BPs should be designed to reveal the potential adverse effects of the drug candidate on the function of CV, central nervous, and respiratory systems. Guidelines S7A and S7B specifically indicate that SP evaluation of BP can be performed using dedicated studies or an integrated approach where SP parameters are measured in toxicity studies, including QTc prolongation evaluation (a surrogate for assessment of torsades de pointes or pro-arrhythmic liability).

The potential undesirable effect of every drug candidate on the CV, central nervous, and respiratory systems, referred to as the "core battery" (ICH S7A), should be assessed prior to first-in-human administration. In addition, follow-up studies may be needed to allow for better understanding of the effects of drug candidates on these systems. Supplemental SP studies may also be needed to assess the effects of drug candidates on other systems such as renal and gastrointestinal (GI), if effects on these systems are suspected based on the class of the drug candidates being tested and/or target liability assessment, e.g., target expression in the particular organ. The core battery studies should be performed in compliance with the good laboratory practice (GLP); however, "follow-up and supplemental studies should be conducted in compliance with GLP to the greatest extent feasible" (Section 2.11, ICH S7A 2000). These SP studies can be either stand-alone or SP parameters assessment could be integrated into toxicity studies. However, the interrogation of SP parameters in a toxicity study should follow the same standards applied to dedicated studies, according to a recent FDA Guidance for Industry (Questions and Answers on ICH M3(R2) Guideline) (Section E, FDA 2013b).

There are circumstances where SP assessment may not be required. These include therapeutics being developed for topical application (dermal or ocular) where the systemic exposure is expected to be low (Section 2.9, ICH S7A 2000). In addition, novel cytotoxic agents being developed for advanced cancer do not require dedicated SP studies, unless there is a cause for concern (Section II B, ICH S9 2010).

Although ICH S6(R1) guideline principals may be applicable to recombinant DNA protein vaccines, SP evaluation of vaccines is not required based on the recommendation of the World Health Organization (WHO) unless "nonclinical and/or human clinical studies suggest that the vaccine my affect physiological functions" (WHO 2003). In that case, SP parameters should be incorporated into toxicity studies (WHO 2003; 2013). This recommendation has been adopted by the FDA (Sun et al. 2012); however, European Union and Japanese regulatory agencies recommend that the effects of vaccines on CV, central nervous, and respiratory systems functions be assessed during repeat-dose toxicity studies before first-in-human exposure (Sun et al. 2012). There are not many reports that describe the SP evaluation of a vaccine, but a report on the activity of an experimental protein-based cancer vaccine [CHP-NY-ESO-1 peptide vaccine—consisting of a recombinant protein of the cancer antigen NY-ESO-1 and a polysaccharide-based delivery system (cholesteryl pullulan)] does provide some insight. In a traditional battery of in vitro and in vivo SP studies, the results indicated that the vaccine did not inhibit hERG channel function and had no effect on vital functions after acute administration, which indicated that this vaccine product had very low potential for altering the CV, central nervous, and respiratory systems (Harada et al. 2008).

For detailed information on safety assessment of vaccines (prophylactic and therapeutic), readers are referred to reviews on this topic (Lebron et al. 2005; Sun et al. 2012; Matsumoto et al. 2014). SP for bacterial- or mammalian cell-derived oligonucleotides is typically evaluated during repeat-dose toxicity studies (Dixit et al. 2010; Kim et al. 2014); however, recommendations and strategies that can be used to assess these unique agents have been developed by the SP subcommittee of the Oligonucleotide Safety Working Group (Schubert et al. 2012; Berman et al. 2014).

4 Choice of Test Species

Choice of the test species is a critical step in the evaluation of the nonclinical safety of BPs. The most important factor in choosing a test species is whether the intended therapeutic target (receptor, channel, etc.) is present in a given species and whether the molecular identity and transduction mechanisms of the target is similar to that in humans. SP assessment of SMs is performed in rodents and non-rodents (Table 1). In contrast, SP assessment of BPs in rodent may not be possible because rodents do not always express orthologue of the human target, or BPs have little or no pharmacological activity against the rodent orthologue. For this reason, nonhuman

primates (NHPs) are the primary species for assessing the safety of BPs (Bussiere 2008). This is supported by a recent pharma-wide survey of SP practices (Authier et al. 2013).

5 In Vitro Safety Pharmacology Profiling

In vitro SP profiling of SM drug candidates, using either binding or cellular functional assays, is typically performed early in the drug development process to allow for selection of a drug candidate with lower potential for off-target activity or side-effect liability (Bowes et al. 2012). Because of the high target selectivity, BPs are not expected to have significant off-target activities; thus, these agents are not typically profiled for secondary pharmacological activity. Two current examples that support the hypothesis that BPs have low off-target potential were identified in an examination of drug approvals. In our survey of approved BPs (Table 2), we found that one peptibody and one monoclonal antibody were profiled pharmacologically for off-target activity. Using conventional methods, romiplostim (NPLATE®, 59,000 Da) and adalimumab (HUMIRA®, ~148,000 Da) were tested in panels containing 63–68 receptors, enzymes, and ion channels and found to be devoid of any significant "off-target" activity, which indicates that these agents were highly selective for their molecular targets.

We reported the receptor profile of an investigational pegylated (polyethylene glycol-20) peptide (~24,000 Da) that was evaluated for potential off-target interactions with 151 receptors, enzymes, ion channels, and transporters. The results indicated eight hits (a "hit" being defined as >50 % inhibition of control-specific binding or enzyme activity at 10 μM), but these were not considered to have relevant safety implications because of their high-exposure multiples relative to the human target potency estimates (Vargas et al. 2013a). In addition, when the PEG moiety alone was tested in the same panel, no significant off-target hits were observed. Based on the current state of knowledge and regulatory practices, in vitro pharmacology profiling of the BPs is not recommended.

6 Cardiovascular System Safety Pharmacology

The nonclinical approach for CV risk assessment of new drug candidates should address potential effects on blood pressure, heart rate, electrocardiography (ECG), and the functional status of critical ion channels (ICH 7A 2000; ICH 7B 2005). To achieve this, various in vitro and in vivo methods can be used. In general, the approach for BPs and SMs is similar; however, there are differences. The first parameter evaluated in the CV risk assessment of SMs is their potential inhibitory effect on hERG channel function using a voltage-clamp assay. Performing this assay for BPs is not considered appropriate because large proteinaceous molecules are not expected to pass though the plasma membrane to gain access to the channel pore, nor are they likely to interact with the "toxin-binding site" on the

extracellular surface of the hERG channel. Recent in vitro studies using anti-hERG-specific antibodies show that these antibodies do not inhibit the function of the channel because they do not bind to key epitopes near the external pore region like BeKm-1 (Qu et al. 2011). Therefore, performing a hERG assay with BPs is considered irrelevant and is not recommended (Vargas et al. 2008).

Blood pressure, heart rate, and the ECG can be assessed in conscious freely moving rodents, dogs, and NHPs using telemetry. For SMs, which typically have a short half-life, this is done using a crossover design where each animal receives every treatment and serves as its own control in a single-dose study. This study design is not appropriate for BPs because of their long half-life. For this reason, integration of CV parameters into repeat-dose toxicity studies with BPs is an appropriate approach which also has the advantage of reducing the overall number of animals. Jacket-based external telemetry (JET™) is the method of choice for collection of high-quality heart rate and ECG parameters from unrestrained animals, particularly NHPs, which are often the species of choice for safety assessment of BPs (Chui et al. 2009, 2011; Guth et al. 2009; Derakhchan et al. 2011, 2014). Performed appropriately, this method allows for collection of parameters during non-rodent toxicity studies that are of comparable quality to those collected in unrestrained large animals using implant telemetry (Chui et al 2009; Derakhchan et al. 2014). The critical aspect of JET™ is the acclimation of the animals to the jacket, which is especially important for NHPs. The acclimation process generally involves multiple sessions of increasing time to allow the animals to become accustomed to the jacketing process and to wearing the jacket. Because acclimation is not long-lasting, it should be conducted as close to the collection period as possible (Derakhchan et al. 2014).

For BPs, hemodynamic parameters such as arterial blood pressure can be assessed using implant telemetry in NHPs (Santostefano et al. 2012). Alternatively, arterial pressure can be measured directly using JET™ with the blood pressure option (JET-blood pressure; McMahon et al. 2010). Implanted telemetry has the advantage of not requiring acclimation to handling and wearing of a jacket, but time is needed to allow the animals to recover from the implantation surgical procedure. Either method (implant telemetry or JET-blood pressure) allows for the collection of arterial pressure data from unrestrained animals for short or long durations which are well suited to characterize the hemodynamic profile of BPs (McMahon et al. 2010; Kaiser et al. 2010). Another method under development for measurement of blood pressure is high-definition oscillometry (Schmelting et al. 2009). At this time, there are many issues with this method, which may limit its usefulness as a sensitive SP tool. These include the inability to detect small changes in blood pressure, inaccurate blood pressure and heart rate due to stress from restraint, and limitation to a single-point measurement as compared to continuous measurements provided by other methods (Kurtz et al. 2005; Wernick et al. 2012).

Cardiac function can also be evaluated directly using echocardiographic imaging (Tsusaki et al. 2005; Hanton et al. 2008) or by measuring left ventricular pressure as an index of cardiac inotropy (Sarazan et al. 2011). The main advantage of the echocardiography method is that the same functional and structure parameters can

be evaluated in both animals and humans noninvasively, which allows for direct comparison of the same endpoints and their translation across species. However, unlike continuous monitoring of left ventricular function by telemetry in freely moving conscious animals, echocardiography is a "snapshot" measurement, i.e., at a single time point, which may require chemical restraint (or extensive acclimation of animals if chemical restraint is not used) to ensure the animals remain properly positioned for the time required to obtain quality cardiac images.

There are cases when a dedicated CV telemetry study may be the best option to assess target liability concerns for BPs. These include the presence of the therapeutic target in the CV system (e.g., cardiac myocytes, vascular endothelium, or vascular smooth muscle) or the emergence of CV findings in nonclinical toxicity studies or clinical trials. For example, observation of cardiac dysfunction in patients treated with trastuzumab, a mAb for treatment of breast cancer, prompted the sponsor to perform a long-term telemetry study in the cynomolgus monkey in attempts to model the human cardiac dysfunction (Klein and Dybdal 2003). Likewise, a novel ADC based on trastuzumab was evaluated in a dedicated NHP telemetry study to evaluate potential target-mediated CV effects (Poon et al. 2013).

In general, the risk for QTc prolongation is considered low for BPs. This is supported by the findings from a review of FDA drug approvals from 2008 to 2015 (Hughes 2009, 2010; Mullard 2011, 2012, 2013, 2014, 2015). Among the 57 BPs approved during this period, only 2 BPs had a QT warning on their label (3.5 %; Table 3). In contrast, 17 of 159 SMs approved during the same period had a QT warning (10.6 % Table 4). Likewise, 93 thorough QT (TQT, a dedicated clinical study designed to assess drug-induced changes in the QTc interval) studies were performed as part of the registration requirements for SMs, compared to only 4 TQT studies on a few BPs (e.g., peginesatide acetate, pasireotide, albiglutide, and ramucirumab).

As part of their clinical development, peginesatide acetate, albiglutide, and ramucirumab were assessed for QTc prolongation risk in a valid TQT study, i.e., with a positive control, and each drug had no effect on cardiac repolarization in humans (peak effect: <10 ms). However, pasireotide diaspartate did have a positive QTc signal, which resulted in a QT warning on the label (Drugs@FDA). Given that BPs are generally considered to have a low risk for cardiac ion channel blockade and QTc prolongation, the effect following pasireotide diaspartate administration deserves further review.

The QTc prolongation caused by pasireotide diaspartate may be an example of a target-driven cardiac safety concern. This drug is a synthetic peptide analog of somatostatin and used for treatment of Cushing's disease (Mullard 2013; Drugs@FDA). Nonclinical studies, including hERG function ($IC_{50} > 30$ μM), Purkinje fiber assays, and a NHP telemetry study demonstrated low potential for delayed cardiac repolarization. Despite the nonclinical profile, QTc prolongation was observed in two TQT clinical trials with this agent. The findings from the initial trial were not reported; however, in the pivotal trial, QT prolongation was reported in approximately 6 % of subjects (Clinical Summary, Drugs@FDA). In healthy volunteers given pasireotide subcutaneously, the mean ΔΔQTcI was 13.2 and

Table 3 Biopharmaceuticals (BPs) with a TQT study or a QT warning in the label

Year	Approved drugs	Approved BPs	TQT study	TQT signal	QT warning
2008	24	4	0	0	1
2009	27	7	0	0	0
2010	21	9	0	0	0
2011	35	11	0	0	0
2012	39	11	2[a]	1	1
2013	27	4	0	0	0
2014	41	11	2	0	0
Total	214	57	4	1	2

TQT: thorough QT
[a]These studies were performed on one BP (pasireotide)

Table 4 Small molecule (SM) therapeutics with TQT studies and QT warning in the label

Year	Approved drugs	Approved SMs	TQT study	TQT signal	QT warning
2008	24	20	11	2	2
2009	27	20	7	2	7
2010	21	12	4	1	1
2011	35	24	16	2	3
2012	39	28	15	1	2
2013	27	25	16	0	0
2014	41	30	24	2	2
Total	214	159	93	10	17

TQT: thorough QT

16.1 ms for the 600 and 1,950 μg twice-daily doses, respectively (Breitschaft et al. 2014). As there was no nonclinical evidence for a direct inhibitory effect of pasireotide on the hERG channel or evidence of any change in Purkinje fiber APD, the specific mechanism of the QTc effect is unknown; however, it could be an indirect or secondary effect on cardiac repolarization. For example, pasireotide administration was associated with hyperglycemia and bradycardia, so the delayed cardiac repolarization could be due to treatment-related alterations in autonomic tone and glucose modulation. Another somatostatin analog, octreotide, has also been associated with QTc prolongation (Drugs@FDA), potentially through a similar mechanism as pasireotide.

Other BPs, such as oxytocin, have been associated with cardiac repolarization risk (see crediblemeds.org). Adverse CV effects, including hypotension, elevated heart rate, cardiac arrhythmia, premature ventricular contractions, and QTc prolongation, have been observed in women treated with oxytocin during abortion and cesarean delivery (Charbit et al. 2004; Guillon et al. 2010). In a nonclinical investigation, similar QTc findings were reported in rabbits administered intravenous bolus injection of oxytocin (Uzun et al. 2007). A mechanistic study of recombinant oxytocin on repolarization in rabbit and human ventricular myocytes demonstrated that this peptide did not inhibit hERG channel function or prolong

action potential duration (APD) and QTc intervals in cardiac models, which strongly suggests that the QTc prolongation observed in humans and animals is not mediated through a direct cardiac site of action (Vargas et al. 2013b; Qu et al. 2015). The findings with pasireotide and oxytocin indicate that some BPs can alter cardiac repolarization indirectly; thus, there is a potential for drug–drug interactions with other therapeutics that have QTc prolongation risk (e.g., antiarrhythmics, some antibiotics, etc.).

7 Central Nervous System Safety Pharmacology

Assessment of the effects of drug candidates on CNS function is required prior to first-in-human exposure for both BPs and SMs (ICH S7A 2000). However, the approach for each type of drug modality is different. For SMs, neurobehavioral effects are typically assessed in rodents using neurofunctional methods such as Irwin, modified Irwin, or a functional observational battery (FOB) which include a battery of observational parameters such as home cage and open arena observation and elicited responses such as reflexes to stimuli. BPs are not expected to enter the CNS under normal circumstances because of their physiochemical properties such as large molecular size and the unique features of the bloods brain barrier (BBB) which is designed to prevent chemicals from gaining access to the brain (Misra et al. 2003; Gabathuler 2010; Freeman et al. 2012; Pardridge 2012). Therefore, it is generally accepted that, under normal physiological conditions, BPs are not expected to enter the CNS and affect neurobehavioral function. This notion is supported by the results of an internal survey of incidence of convulsion which indicates that none of the BPs ($N = 11$) caused convulsion whereas 14 % of small molecules caused convulsion. The incidence of convulsions was further confirmed by electroencephalographic studies in either rats or in NHPs which showed evidence of seizure activity for SMs that cause convulsion (Amouzadeh and Vargas 2013; Vargas et al. 2013a). In addition, the label for raxibacumab, a mAb against protective antigen of *Bacillus anthracis* for prophylaxis or treatment of inhalational anthrax in combination with antibacterial drugs, explicitly indicates that it "appears unable to penetrate the CNS until compromise of the BBB during latter stages of anthrax infection" (Drugs@FDA).

To overcome the lack of access of the BPs to the CNS in cases such as brain tumors where a BP could be a beneficial therapy, drug-delivery approaches such as receptor-mediated transcytosis through BBB can be exploited to deliver BPs to the CNS (Yu et al. 2011; Pardridge and Boado 2012; Yi et al. 2014).

Regardless of theoretical and practical aspects, neurobehavioral effects of BPs should be evaluated prior to first-in-human exposure. In cases where the therapeutic target is present in rodents, CNS studies can be performed either in dedicated Irwin/FOB studies or parameters can be integrated into toxicity studies. However, when the choice of species is limited to NHPs, neurobehavioral effects of BPs are typically assessed during repeat-dose toxicity studies as recommended by ICH 7A (2000). The use of an integrated approach has the advantage of being in line

with 3Rs principles, especially the optimization of NHPs use in toxicity studies. Methods have been developed to obtain detailed neurobehavioral data from NHPs beyond basic clinical observations as part of standard toxicity or cardiovascular telemetry studies (Korte et al. 2007; Gauvin and Baird 2008; Moscardo et al. 2010).

A recent report showed that assessing spontaneous locomotor activity in rodents using noninvasive methods based on infrared beam (Actimeter®) or vibration (LABORAS®) is a useful predictor of neurobehavioral effects of drug candidates (Lynch et al. 2011; Golozoubova et al. 2014) that can be used early in the drug development process. Although BPs are not expected to cause significant neurobehavioral effect because of their poor penetration into the CNS under normal circumstances, the possibility of neurobehavioral effects cannot be ruled out. A recent survey of 50 drug candidates for non-CNS indications (and with limited access to CNS) showed that the majority of them affected at least one parameter of the FOB in rats. However, this may not necessarily mean a specific effect on the CNS, but rather secondary behavioral changes due to general drug-induced toxicity or CV effects (Redfern et al. 2005). Therefore, observation of an effect during the neurofunctional evaluation of a BP should be followed by more rigorous testing to determine whether the effect was actually caused by action of the BP on the CNS or an indirect behavioral effect.

8 Respiratory System Safety Pharmacology

Because of their selectivity, BPs are not expected to adversely affect the respiratory system through their potential off-target activity, unless there is a concern about target-based toxicity. This is reflected in a recent publication documenting a lack of adverse respiratory effects in the rat with BPs (Vargas et al. 2013a). However, assessment of respiratory function in nonclinical species is required prior to first-in-human exposure. ICH 7A (2000) guideline states that the "Effects of the test substance on the respiratory system should be assessed appropriately. Respiratory rate and other measures of respiratory function (e.g., tidal volume or hemoglobin oxygen saturation) should be evaluated. Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies" (Section 2.7.3, ICH 7A 2000). Based on the findings from the initial assessment, there may be a need to perform follow-up studies to evaluate "airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH, etc." (Section 2.8.1.3, ICH 7A 2000).

Typically, respiratory system SP assessments of SMs are performed using either head-out or whole-body plethysmography. Parameters such as respiratory rate (RR) and tidal volume (TV) are measured, and many other parameters such as minute volume, peak inspiratory flow (PIF), peak expiratory flow (PEF), enhanced pause (Penh), inspiration time (IT), and expiration time (ET) are derived. Although there are many methods for respiratory function assessment in rodents (Murphy 2002, 2014; Hoffman et al. 2007; Hoymann 2012), such methods have not been validated extensively for large animals such as NHPs. Therefore, respiratory

function assessment of large animals is integrated in toxicity studies and oftentimes is limited to clinical observation during toxicity studies which is not a sensitive method to assess subtle changes in respiratory function. Although respiratory function assessment could be performed during a toxicity study, it requires skilled staff and careful planning to assure that accurate data are collected during maximal drug effect. There may be cases where a more rigorous assessment of the respiratory function such as pulmonary resistance is needed based on the presence of therapeutic target in the lung and/or empirical observation of pulmonary (lung) pathology in toxicity studies of a BP drug candidate. For this, a dedicated respiratory SP study may be needed to assess functional consequences and inform the need for a clinical monitoring strategy. Initial assessment can be performed in NHPs using head-out or whole-body plethysmography (Iizuka et al. 2010; Lawler et al. 2006; Nalca et al. 2010), and follow-up evaluation, such as measurement of pulmonary resistance, can be done in anesthetized animals (Chapman et al. 2005; Skeans et al. 2005; Curran et al. 2008). Recently, airway oscillometry has been reported to be a useful method for noninvasive evaluation of respiratory function in dogs and cynomolgus monkeys (Bassett et al. 2014b). This latter method holds promise to enable a more robust and quantitative evaluation of respiratory function in non-rodent SP and toxicology studies.

9 Follow-Up and Supplemental Safety Pharmacology

Follow-up and supplemental SP studies may be needed based on the findings from "safety pharmacology core battery, clinical trials, pharmacovigilance, experimental in vitro or in vivo studies, or from literature reports" (ICH 7A 2000).

The purpose of follow-up studies is to allow for a further understanding of the findings. These follow-up studies could include "behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory and/or electrophysiology examinations, cardiac output, ventricular contractility, vascular resistance, the effects of endogenous and/or exogenous substances on the cardiovascular responses, airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH, etc." (ICH 7A 2000).

The need for the conduct of supplemental SP studies is informed by findings during nonclinical development when adverse effects on the function of systems other than those in the core battery are observed. These systems include renal/urinary, autonomic, GI, and other organ systems. ICH S7A guideline cites a number of parameters that can be used to assess the effects of drug candidates on the function of these systems. For example, urinary parameters such as volume and electrolyte excretion, GI parameters such as transit time and diarrhea, immunological parameters such immune cell phenotyping, and endocrine parameters such as hormone levels may be used to assess functional changes in these particular organ systems (Section 2.8.2, ICH 7A 2000). These parameters could be assessed either in stand-alone SP or repeat-dose toxicity studies. For example, stand-alone video-EEG can be used to assess seizure liability in rats, dogs, or monkeys (Bassett

et al. 2014a). The approaches for SP assessment of SMs and BPs described above apply to the follow-up and supplemental pharmacology studies as well; thus, supplemental SP endpoints can be evaluated as add-on measurements as part of a repeat-dose toxicity study.

Assessment of abuse and dependency potential of a drug candidate is also needed if it shows CNS activity and is chemically or pharmacologically similar to known drugs of abuse and produces psychoactive effects (Section 15, ICH M3 (R2) 2009). The general nonclinical approach and details for this assessment are described in the guidance issued by FDA (FDA 2010b), the decision tree presented by FDA (Bonson and Sun 2011), and the guideline issued by EMA in 2006. Based on the rationale and empirical findings cited above on the lack of access to CNS by BPs, assessment of abuse and dependency potential of BPs are not warranted. However, in cases where a BP is designed to penetrate the CNS, such assessment may be needed (Yu et al. 2011; Pardridge and Boado 2012).

10 Summary

Safety evaluation of novel BPs begins during target liability assessment when potential adverse effects of modulating a target are identified. The most efficient path to SP evaluation of novel BPs and fulfillment of the regulatory requirements is by integrating the collection of relevant SP parameters into toxicity studies when possible and practical (Redfern et al. 2013; Authier et al. 2013). In specific cases, based on target liability concerns, or the emergence of unanticipated pharmacological or toxicological findings, a dedicated SP study may be needed to address the liability (Santostefano et al. 2012; Klein and Dybdal 2003; Poon et al. 2013). There may also be a need to perform supplemental SP when there is a cause for clinical concern. Thus, the core message is that the SP strategy for BPs will be influenced by many factors and is guided by the need to know whether functional changes in organ systems responsible for vital functions (CV, central nervous, and respiratory systems) are impacted by a BP. In vitro SP profiling of the BPs is not recommended as part of routine screening based on the current state of knowledge and regulatory practices.

11 Future Prospects

The safety assessment of drug candidates is an evolving science. This has been envisaged in the nonclinical guidelines in that they recommend general, rather than specific, approaches to safety assessment. This is because the development of each BP drug candidate is unique (or "fit for purpose") and there is continued improvement in the understanding of the science, technologies, and methods during preclinical drug development.

One of the major improvements in nonclinical SP assessment of the drug candidate is the use of telemetry systems which allow for extended evaluation of

CV parameters in unrestrained rodents and non-rodents. An example is the evaluation of the effects of drug candidates on the CV system using either a jacket or an implanted device to collect high-quality ECG or blood pressure data for extended periods.

There are also emerging methodologies for improved assessment of respiratory, central nervous, GI, and renal systems. However, many of these methods are not part of standard practice, because they have not been widely studied or validated. Jacket-based inductive plethysmography (Ingram-Ross et al. 2012) and airwave oscillometry (Bassett et al. 2014b) are noninvasive methods that can be used to monitor respiratory function quantitatively in large animals in either stand-alone or integrated SP studies. Assessing spontaneous locomotor activity in rodents using noninvasive methods based on infrared beam (Actimeter®) or vibration (LABORAS®) has been shown to be a useful predictor of neurobehavioral effects of drug candidates (Lynch et al. 2011). This technique can be used to evaluate the potential of BPs to cause neurobehavioral effects early in the drug development process if the target is expressed in the test species. New technologies such as SmartPill® may be useful to assess effects of BPs on GI function (transit time), and biomarkers of dysfunction (e.g., blood urea nitrogen or creatinine) or injury (e.g., KIM-1) could be used as an indicator of renal toxicity of BPs, but will require further testing and validation in large animal species, especially the NHPs.

An alternative approach to CNS and respiratory SP assessment in rats is performing these studies using the same animals, but tested in a sequential manner. During the first phase of the study, neurobehavioral effects of the test compound are assessed. Then, an interim period of at least 1 week is allowed for clearance of the test compound during which animals are gradually acclimated to the plethysmography apparatus. During the second phase, the effect of test compound on the respiratory system is evaluated. This alternative approach is most appropriate for SMs, which typically have a short half-life or BPs with short half-life such as peptides that show activity in rodents. Major advantages of this approach are substantial reduction in the number of animals and the potential for lower overall study costs.

References

Amouzadeh HR, Vargas HM (2013) Safety pharmacology assessment of biopharmaceuticals. In: Vogel HG et al (eds) Drug discovery and evaluation: safety and pharmacokinetic assays. Springer, Berlin, pp 555–560

Authier S, Vargas HM, Curtis MJ et al (2013) Safety pharmacology investigations in toxicology studies: an industry survey. J Pharmacol Toxicol Methods 68:44–51

Bassett L, Troncy E, Pouliot M et al (2014a) Telemetry video-electroencephalography (EEG) in rats, dogs and non-human primates: methods in follow-up safety pharmacology seizure liability assessments. J Pharmacol Toxicol Methods. doi:10.1016/j.vascn.2014.07.005

Bassett L, Troncy E, Pouliot M et al (2014b) Non-invasive measure of respiratory mechanics and conventional respiratory parameters in conscious large animals by high frequency airwave oscillometry. J Pharmacol Toxicol Methods 70:62–65

Berman CL, Cannon K, Cui Y et al (2014) Recommendations for safety pharmacology evaluations of oligonucleotide-based therapeutics. Nucleic Acid Ther 24:291–301

Bonson K, Sun S (2011) FDA's draft decision tree for assessment of abuse potential. Presented at the science of abuse liability assessment, legacy hotel, Rockville, MD, Nov 10, 2011

Bowes J, Brown AJ, Hamon J et al (2012) Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. Nat Rev Drug Discov 11:909–922

Breitschaft A, Hu K, Darstein C et al (2014) Effects of subcutaneous pasireotide on cardiac repolarization in healthy volunteers: a single-center, phase I, randomized, four-way crossover study. J Clin Pharmacol 54:75–86

Bussiere JL (2008) Species selection considerations for preclinical toxicology studies for biotherapeutics. Expert Opin Drug Metab Toxicol 4:871–877

Chapman RW, Skeans S, Lamca J et al (2005) Effect of histamine, albuterol and deep inspiration on airway and lung tissue mechanics in cynomolgus monkeys. Pulm Pharmacol Ther 18:243–249

Charbit B, Samain E, Albaladejo P et al (2004) QT interval prolongation after oxytocin bolus during surgical induced abortion. Clin Pharmacol Ther 76:359–364

Chui RW, Fosdick A, Conner R et al (2009) Assessment of two external telemetry systems (PhysioJacket and JET) in Beagle dogs with telemetry implants. J Pharmacol Toxicol Methods 60:58–68

Chui RW, Derakhchan K, Vargas HM (2011) Long-term assessment of non-human primate ECG using jacketed external telemetry (JET): evaluation of heart rate and QTc interval variation over 6 months of observation. J Pharmacol Toxicol Methods 64:e45

Curran AK, Skeans S, Landers D et al (2008) Differential effects of dexamethasone on the proximal and distal lung response to antigen challenge in allergic cynomolgus monkeys. J Asthma 45:377–381

Derakhchan K, Chui RW, Vargas HM (2011) Evaluation of cardiac conduction disturbances using jacketed external telemetry (JET) in conscious non-human primates. J Pharmacol Toxicol Methods 64:e46

Derakhchan K, Chui RW, Stevens D et al (2014) Detection of QTc interval prolongation using jacket telemetry in conscious non-human primates: comparison with implant telemetry. Br J Pharmacol 171:509–522

DiMasi JA, Feldman L, Seckler A et al (2010) Trends associated with new drug development: success rates for investigational drugs. Clin Pharmacol Ther 87:272–277

Dixit R, Iciek LA, McKeever K et al (2010) Challenges of general safety evaluations of biologics compared to small molecule pharmaceuticals in animal models. Expert Opin Drug Discov 5:79–94

Drugs@FDA SANDOSTATIN® (1988), MYLOTARG® (2000), ADCETRIS® (2011) OMONTYS® (2012), SIGNIGOR® (2012), RAXIBACUMAB® (2012), and KADCYLA® (2013) (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?CFID=19379093&CFTOKEN=25eb8af4655376b-DCB09EA0-0293-9EC5-1C37C084658F1067)

FDA (2009) FDA statement on the voluntary withdrawal of RAPTIVA® From the U.S. Market (http://www.fda.gov/newsevents/newsroom/pressannouncements/2009/ucm149561.htm)

FDA (2010a) Gemtuzumab Ozogamicin (MYLOTARG®) ((http://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm216790.htm)

FDA (2010b) Guidance for industry: assessment of abuse potential of drugs. (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm198650.pdf)

FDA (2013a) Affymax and Takeda Announce a Nationwide Voluntary Recall of All Lots of OMONTYS® (peginesatide) Injection (http://www.fda.gov/safety/recalls/archiverecalls/2013/ucm340893.htm)

FDA (2013b) Guidance for Industry (2013) M3(R2) Nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals – questions and answers (R2). Available at FDA.gov (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292340.pdf)

Frankel SR, Baeuerle PA (2013) Targeting T cells to tumor cells using bispecific antibodies. Curr Opin Chem Biol 17:385–392
Freeman GB, Lin JC, Pons J et al (2012) 39-Week toxicity and toxicokinetic study of ponezumab (PF-04360365) in cynomolgus monkeys with 12-week recovery period. J Alzheimers Dis 28:531–541
Gabathuler R (2010) Approaches to transport therapeutic drugs across the blood brain barrier to treat brain diseases. Neurobiol Dis 37:48–57
Gauvin DV, Baird TJ (2008) A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. J Pharmacol Toxicol Methods 58:88–93
Giezen TJ, Mantel-Teeuwisse AK, Straus SM et al (2008) Safety-related regulatory actions for biological approved in the United States and the European Union. JAMA 300:1887–1896
Golozoubova V, Brodersen TK, Klastrup S et al (2014) Repeated measurements of motor activity in rats in long-term toxicity studies. J Pharmacol Toxicol Methods 70:241–245. doi:10.1016/j.vascn.2014.06.007
Guillon A, Leyre S, Remerand F et al (2010) Modification of Tp-e and QTc intervals during caesarean section under spinal anaesthesia. Anaesthesia 65:337–342
Guth BD, Bass AS, Briscoe R et al (2009) Comparison of electrocardiographic analysis for risk of QT interval prolongation using safety pharmacology and toxicology studies. J Pharmacol Toxicol Methods 60:107–116
Hanton G, Eder V, Rochefort G, Bonnet P et al (2008) Echocardiography, a non-invasive method for the assessment of cardiac function and morphology in preclinical drug toxicology and safety pharmacology. Expert Opin Drug Metab Toxicol 4(6):681–696
Harada N, Hoshiai K, Takahashi Y et al (2008) Preclinical safety pharmacology study of a novel protein-based cancer vaccine CHP-NY-ESO-1. Kobe J Med Sci 54:E23–E34
Hoffman W, Kallman MJ, Sgro M (2007) Respiratory safety pharmacology. In: Sietsma WK, Schwen R (eds) Nonclinical drug safety assessment: practical considerations for successful registration. FDA News, Falls Church, VA, Chapter 7
Hoymann HG (2012) Lung function measurements in rodents in safety pharmacology studies. Front Pharmacol 3:156
Hughes B (2009) 2008 FDA drug approval. Nat Rev Drug Discov 8:93–96
Hughes B (2010) 2009 FDA drug approval. Nat Rev Drug Discov 9:89–92
ICH M3(R2) (2009) Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. Available at ICH.org (Retrieved July 2014).
ICH S6(R1) (2011) Preclinical safety evaluation of biotechnology-derived biopharmaceuticals. Available at ICH.org (Retrieved July 2014).
ICH S7A (2000) Safety pharmacology studies for human biopharmaceuticals. Available at ICH.org (Retrieved July2014).
ICH S7B (2005) The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human biopharmaceuticals. Available at ICH.org (Retrieved July 2014).
ICH S9 (2010) Nonclinical evaluation for anticancer pharmaceuticals. Available at ICH.org (Retrieved July 2014)
Iizuka H, Sasaki K, Odagiri N et al (2010) Measurement of respiratory function using whole-body plethysmography in unanesthetized and unrestrained non-human primates. J Toxicol Sci 35:863–870
Ingram-Ross JL, Curran AK, Miyamoto M et al (2012) Cardiorespiratory safety evaluation in non-human primates. J Pharmacol Toxicol Methods 66:114–124
Kaiser RA, Erwin R, Tichenor SD et al (2010) Integration of cardiovascular safety pharmacology endpoints into general toxicology studies. J Pharmacol Toxicol Methods 62:e30
Kim TW, Kim KS, Seo JW et al (2014) Antisense oligonucleotides on neurobehavior, respiratory and cardiovascular function and hERG channel current studies. J Pharmacol Toxicol Methods 69:49–60

Klein PM, Dybdal N (2003) Trastuzumab and cardiac dysfunction: update on preclinical studies. Semin Oncol 30:49–53
Korte S, Fuchs A, Weinbauer GF et al (2007) Modified Irwin test as diagnostic tool to monitor neurobehavioral changes in monkeys. J Pharmacol Toxicol Methods 56:e47
Kurtz TW, Griffin KA, Bidani AK et al (2005) Recommendations for blood pressure measurement in humans and experimental animals. Hypertension 45:299–310
Lawler JV, Endy TP, Hensley LE et al (2006) Cynomolgus macaque as an animal model for severe acute respiratory syndrome. PLoS Med 3:e149
Lebron JA, Wolf JJ, Kaplanski CV, Ledwith BJ (2005) Ensuring the quality, potency and safety of vaccines during preclinical development. Expert Rev Vaccines 4(6):855–866
Lynch JJ III, Castagne V, Moser PC et al (2011) Comparison of methods for the assessment of locomotor activity in rodent safety pharmacology studies. J Pharmacol Toxicol Methods 64:74–80
Marafino BJ, Pugsley MK (2003) Commercial development considerations for biotechnology-derived therapeutics. Cardiovasc Toxicol 3:5–12
Matsumoto M, Komatsu S, Tsuchimoto M et al (2014) Considerations for non-clinical safety studies of therapeutic peptide vaccines. Reg Pharmacol Toxicol 70(1):254–60. doi:10.1016/j.yrtph.2014.06.029)
McMahon C, Mitchell AZ, Klein JL et al (2010) Evaluation of blood pressure measurement using a miniature blood pressure transmitter with jacketed external telemetry in Cynomolgus monkeys. J Pharmacol Toxicol Methods 62:127–135
Misra A, Ganesh S, Shah SP (2003) Drug delivery to the central nervous system: a review. J Pharm Pharm Sci 6:252–273
Moscardo E, McPhie G, Fasdelli N et al (2010) An integrated cardiovascular and neurobehavioral functional assessment in the conscious telemetered cynomolgus monkey. J Pharmacol Toxicol Methods 62:95–106
Mullard A (2011) 2010 FDA drug approvals. Nat Rev Drug Discov 10:82–85
Mullard A (2012) 2011 FDA drug approvals. Nat Rev Drug Discov 11:91–94
Mullard A (2013) 2012 FDA drug approvals. Nat Rev Drug Discov 12:87–90
Mullard A (2014) 2013 FDA drug approvals. Nat Rev Drug Discov 13:85–89
Mullard A (2015) 2014 FDA drug approvals. Nat Rev Drug Discov 14:77–81
Murphy DJ (2002) Assessment of respiratory function in safety pharmacology. Fundam Clin Pharmacol 16:183–196
Murphy DJ (2014) Optimizing the use of methods and measurement endpoints in respiratory safety pharmacology. J Pharmacol Toxicol Methods 70(3):204–209. doi:10.1016/j.vascn.2014.03.174)
Nalca A, Livingston VA, Garza NL et al (2010) Experimental infection of cynomolgus macaques (Macaca fascicularis) with aerosolized monkeypox virus. PLoS One 5(e1):2880
Pardridge WM (2012) Drug transport across the blood-brain barrier. J Cereb Blood Flow Metab 32:1959–1972
Pardridge WM, Boado RJ (2012) Reengineering biopharmaceuticals for targeted delivery across the blood–brain barrier. Methods Enzymol 503:269–292
Perez H, Cardarelli PM, Deshpande S et al (2014) Antibody–drug conjugates: current status and future directions. Drug Discov Today 19:869–881
Poon KA, Flagella K, Beyer J et al (2013) Preclinical safety profile of trastuzumab emtansine (T-DM1): mechanism of action of its cytotoxic component retained with improved tolerability. Toxicol Appl Pharmacol 273:298–313
Qu Y, Fang M, Gao B et al (2011) BeKm-1, a peptide inhibitor of hERG potassium currents, prolongs QTc interval in isolated rabbit hearts. J Pharmacol Exp Ther 337:2–8
Qu Y, Fang M, Gao B et al (2015) Oxytocin does not directly alter cardiac repolarization in rabbit or human cardiac myocytes. Pharmacol Res Perspect 3(1):e00102. doi:10.1002/prp2.102

Redfern WS, Strang I, Storey S et al (2005) Spectrum of effects detected in the rat functional observational battery following oral administration of non-CNS targeted compounds. J Pharmacol Toxicol Methods 52:77–82

Redfern WS, Ewart LC, Lainee P et al (2013) Functional assessment in repeat-dose toxicity studies: the art of the possible. Toxicol Res 2:209–234

Santostefano MJ, Kirchner J, Vissinga C et al (2012) Off-target platelet activation in macaques unique to a therapeutic monoclonal antibody. Toxicol Pathol 40:899–917

Sarazan RD, Mittelstadt S, Guth B et al (2011) Cardiovascular function in nonclinical drug safety assessment: current issues and opportunities. Int J Toxicol 30:272–286

Schmelting B, Neihoff M, Egner B et al (2009) High-definition oscillometry: a novel technique for non-invasive blood pressure monitoring in the cynomolgus monkey. J Med Primatol 38: 293–301

Schubert D, Levin AA, Kornbrust D, Berman CL et al (2012) The Oligonucleotide Safety Working Group. Nucleic Acid Ther 22(4):211–213

Shimamoto G, Gegg C, Boone T et al (2012) Peptibodies: A flexible alternative format to antibodies. mAbs 4:586–591

Skeans S, Lamca J, House A et al (2005) Airway closure after antigen challenge in cynomolgus monkeys: effect of the histamine H_1 receptor antagonist, chlorpheniramine maleate. Int Arch Allergy Immunol 137:37–44

Sun Y, Gruber M, Matsumoto M (2012) Overview of global regulatory toxicology requirements for vaccines and adjuvants. J Pharmacol Toxicol Methods 65:49–57

Tsusaki H, Yonamine H, Tamai A et al (2005) Evaluation of cardiac function in primates using real-time three-dimensional echocardiography as applications to safety assessment. J Pharmacol Toxicol Methods 52:182–187

Uzun M, Yapar K, Uzlu E et al (2007) QT interval prolongation and decreased heart rates after intravenous bolus oxytocin injection in male and female conscious rabbits. Gen Physiol Biophys 26:168–172

Vargas HM, Bass AS, Breidenbach A et al (2008) Scientific review and recommendations on preclinical cardiovascular safety evaluation of biologics. J Pharmacol Toxicol Methods 58:72–76

Vargas HM, Amouzadeh HR, Engwall MJ (2013a) Nonclinical strategy considerations for safety pharmacology: evaluation of biopharmaceuticals. Expert Opin Drug Saf 12(1):91–102

Vargas HM, Fang M, Gao BX, et al. (2013b) Oxytocin does not prolong repolarization in the isolated rabbit heart. Presented at the 13th annual meeting of safety pharmacology society, Rotterdam, September 16–19

Wernick MB, Hopfner RM, Francey T et al (2012) Comparison of arterial blood pressure measurements and hypertension scores obtained by use of three indirect measurement devices in hospitalized dogs. J Am Vet Med Assoc 240:962–968

WHO (2003) Guidelines on nonclinical evaluation of vaccines. Available at WHO.int

WHO (2013) Guidelines on nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. (http://www.who.int/biologicals/areas/vaccines/ADJUVANTS_Post_ECBS_edited_clean_Guidelines_NCE_Adjuvant_Final_17122013_WEB.pdf)

Yi X, Manickam DS, Byrnskikh A et al (2014) Agile delivery of protein therapeutics to CNS. J Control Release 190:637–663. doi:10.1016/j.jconrel.2014.06.017

Yu YJ, Zhang Y, Kenrick M et al (2011) Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Sci Transl Med 3:1–8