Biomolecules Versus Smaller Chemicals in Toxicology: ICH, EU, and US Recommendations

Manfred Ruthsatz, Carlo Chiavaroli, M. A. Cassar, and Emmanuelle M. Voisin

Contents

Abstract

For many life science professionals, biological products represent the cutting edge of medical research and are the smartest means to target and treat a variety of disease and conditions for which the current treatments are still unsatisfactory. In contrast to small molecule drugs, including new chemical entities (NCEs),

M. Ruthsatz (✉)
Nutrition+HealthCARE, Vevey, Switzerland
e-mail: ManfredRuthsatz@gmail.com

C. Chiavaroli · M. A. Cassar
Voisin Consulting Life Sciences, Lausanne, Switzerland
e-mail: chiavaroli@voisinconsulting.com; cassar@voisinconsulting.com

E. M. Voisin
Voisin Consulting Life Sciences, Boulogne (Paris), France
e-mail: voisin@voisinconsulting.com; vcparis@voisinconsulting.com

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biomolecules (also called biologics, biologicals, biopharmaceuticals, or biotechnology-derived pharmaceuticals) are complex macromolecules, sometimes occurring as mixtures that are not easily identified or fully characterized. Nevertheless, due to the rapid development of biotechnology in the last three decades, the number of approved biomolecules is increasing at a faster rate than it is the case for new chemical entities. Biologicals are usually highly specific for a target, are more heat sensitive and susceptible to microbial contamination, and are likely antigenic. Thus, the quality and security testing of biologicals is becoming increasingly important. This updated chapter compares the evolving regulatory environment relevant for biomolecules, with a typical "case-by-case" development program versus NCEs, which are generally developed according to a more standard "classical" manner.

Keywords

Chemical entities · Small molecules · Biomolecules · Biologicals · Toxicology

Introduction

Biotechnology-derived pharmaceuticals (biopharmaceuticals, biologicals, or more simply biologics) are defined as products derived from characterized cells through the use of various expression systems including bacteria, yeast, insect, plant, and mammalian cells. The active substances include proteins and peptides, their derivatives, and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals.

Conversely, a NCE (new chemical entity) can be defined as a novel drug substance obtained by chemical change or synthesis and not yet approved for the prevention or treatment of human diseases.

In the EU, all human medicines derived from biotechnology and other high-tech processes are evaluated by the European Medicines Agency (EMA) via the centralized procedure (Notice to Applicants 2016, Vol. 2A).

In the USA, the regulatory evaluations are made either by the Center for Biologics Biopharmaceuticals Evaluation and Research (CBER) or by the Center for Drug Evaluation and Research (CDER). The mission of the CBER at the Food and Drug Administration (FDA) is to ensure the safety, purity, potency, and effectiveness of biological products including vaccines, allergenics, blood and blood products, and cells, tissues, and gene therapies for the prevention, diagnosis, and treatment of human diseases, conditions, or injury. As part of the FDA, the Center for Drug Evaluation and Research (CDER) regulates over-the-counter (OTC) and prescription drugs, including biological therapeutics and generic drugs.

In order to reduce regional discrepancies, the International Conference of Harmonization (ICH) has contributed to a significant global standardization of test conditions and regulatory approval of drugs for quality (ICH Q guidelines), safety

(ICH S guidelines), efficacy (ICH E guidelines), and multidisciplinary (ICH M guidelines). ICH guidelines consider current practices from participating countries and provide a unified view, intended to facilitate mutual acceptance of data and clarify key issues. However, they are typically not statutory by definition and hence not usually legally binding or directly enforceable. The aim of this updated review is to clarify the nonclinical and toxicological regulatory differences encountered when developing NCEs or biologicals, not only taking into account ICH perspectives but also considering regional differences between the EU and US regulations.

Biomolecules Versus Smaller Chemicals in Toxicology

ICH Guidelines

The major ICH guidelines and related Questions and Answers (Q&As) applicable for nonclinical development (ICH S and some ICH M guidelines) are listed in Table 1.

All ICH guidelines listed in this document and their corresponding associated files can be freely downloaded on the https://www.ich.org/ website.

In contrast with the development of NCEs, the list comprises a unique guideline, ICH S6(R1), to address the regulatory environment for the nonclinical development of all biologics (see also Baumann 2009 for a "fundamental review" on nonclinical development of biologics). It is crucial to follow the recommendations of ICH S6 (R1) to achieve the three main goals of nonclinical safety evaluation which are to identify (1) an initial safe dose and subsequent dose escalation schemes in humans, (2) potential target organs for toxicity and for the study of whether such toxicity is reversible, and (3) safety parameters for clinical monitoring. Complying with the recommendations of ICH S6(R1) may, however, still be insufficient to fully predict life-threatening adverse events in man, as discussed below.

It is also important to mention that sometimes assessments may vary between authorities. As an example based on our experience, despite the clear mention in ICH S6(R1) that this guidance may also apply to oligonucleotide drugs, oligonucleotides are usually considered as biotechnology-derived pharmaceuticals by the EMA (following ICH S6(R1), while in the US oligonucleotides are rather evaluated as small molecules by the FDA (following ICH M3(R2)). Therefore, the recommendation for oligonucleotides would be to prepare a nonclinical package that would comply to both guidelines for a worldwide drug development and to request a scientific advice meeting with a regulatory agency to discuss the relevance of the nonclinical development program.

The Regulatory Environment to Initiate First-in-Human Studies

Despite the conduct of a nonclinical development plan in line with the ICH regulations, two dramatic clinical cases necessitated the revision of the regulatory environment for First-in-Human trials in the EU.

Table 1 List of ICH guidelines to assess the safety of NCEs and biotechnology-derived pharmaceuticals

ICH Guideline	Topic (date of coming into force)
M3(R2)	Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (re-amended in 2009). Q&As to ICH M3(R2)
M7(R1)	Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (amended in 2017). ICH M7 (R2) is in preparation
S1A	The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals (1995). ICH S1 as a whole is under revision. The goal is to define when 2-year rat carcinogenicity studies are mandatory
S1B	Testing for Carcinogenicity of Pharmaceuticals (1997)
S1C(R2)	Dose Selection for Carcinogenicity Studies of Pharmaceuticals (amended in 2008)
S2(R1)	Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (amended in 2012)
S3A	Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (1994). Q&As to ICH S3A: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies. Focus on Microsampling (2017)
S3B	Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies (1994)
S4A	Duration of Chronic Toxicity Testing in Animals: Rodent and Non-rodent Toxicity Testing (1998)
S5(R2)	Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (re-amended in 2000, a new version, R3, is in preparation)
S6(R1)	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (amended in 2011)
S7A	Safety Pharmacology Studies for Human Pharmaceuticals (2000)
S7B	Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (2005). Q&As Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrythmic Potential (in preparation 2018)
S8	Immunotoxicity Studies for Human Pharmaceuticals (2005)
S9	Nonclinical Evaluation for Anticancer Pharmaceuticals (2009). Q&As: Nonclinical Evaluation for Anticancer Pharmaceuticals (2018)
S10	Photosafety Evaluation of Pharmaceuticals (2013)
S11	Nonclinical Safety Testing in Support of Development of Paediatric Medicines (2018, step 2b)

1. In 2006, the press (Suntharalingam 2006) reported that failure to select a safe starting dose in humans at the early clinical stage with the CD28 super-agonist monoclonal antibody TGN1412 led to serious toxicity including multi-organ failure in six healthy volunteers. EMA subsequently issued recommendations and a guideline for the safe and rational conduct of clinical trials (EMEA/CHMP/SWP/294648/2007).
   Therefore, since 2007, both the NOAEL dose (No-Observed Adverse Effect Level, which is related to the "toxicological" effects of a drug) and MABEL

dose (Minimum Anticipated Biological Effect Level, which reflects rather the "pharmacological" effect of the drug) should be determined by the Sponsor. The lower of these two doses should be considered for selecting the starting dose in humans. Even if the European guideline was implemented for both NCEs and biologics, the acquired experience demonstrates that the MABEL dose should be especially considered, and receptor occupancy at this dose calculated, when the drug under development is a biological super-agonist molecule and when its mechanism of action suggests it can lead to uncontrolled enzymatic or cytokine cascade reactions.

2. In 2016, a Phase 1 trial of BIA 10-2474, a fatty acid amide hydrolase inhibitor, led to one death and caused serious neurological damage in few other healthy volunteers (Singh 2018).
   As a consequence, in 2017 the EMA updated the 2007 clinical guideline to further assist stakeholders in the transition from nonclinical to early clinical development and to identify factors influencing risk for new investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1, 2017). This new version focused on the maximum exposure of healthy volunteers that should be within the estimated human pharmacodynamics dose range. This guideline, applicable to all new chemicals and biologics, somehow supersedes in the EU the previous US 2005 Guidance for Industry "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." The new 2017 version of the European clinical guideline mentions the PAD (Pharmacologically Active Dose) and/or ATD (Anticipated Therapeutic Dose) range that must be estimated in humans. In addition, the calculation of the MABEL, PAD, and/or ATD should consider target binding and receptor occupancy studies in vitro in target cells from human and the relevant animal species. Whenever possible, all relevant data should be integrated in a suitable modeling approach for the determination of the MABEL, PAD, and/or ATD.

   The bottom line is that the test program to be performed when developing such biologics should be adapted ("case by case") to the properties of the product in development and may be fundamentally different from the toxicological and more "conventional" or "classical" program designed to develop small molecules (see Table 2).

Comparison Between the Nonclinical Assessments of Biologics and New Chemical Entities

Table 2 compares the main nonclinical activities (pharmacology, pharmacokinetics, toxicology) to be performed with biologics and new chemical entities.

Table 2 Nonclinical activities to be conducted for biologics vs. NCEs

Nonclinical activities (guidelines)	Biologics (ICH S6 applies)	New chemical entities
Pharmacology		
Primary pharmacodynamics (ICH M3(R2))	Yes, in vitro and in vivo, in at least a relevant species/model (case by case: humanized model if necessary)	Yes, in appropriate in vitro and in vivo models
Secondary Pharmacodynamics (ICH M3 (R2))	If relevant, in appropriate in vitro and in vivo models	If relevant, in appropriate in vitro and in vivo models
Safety pharmacology (ICH M3(R2), ICH 7A & B)	Yes (CNS, cardiovascular and respiratory systems, other systems if necessary)	Yes (CNS, cardiovascular and respiratory systems, other systems if necessary)
Pharmacokinetics and Toxicokinetics		
Analytical methods and Validation reports (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. 2)	Yes	Yes
Absorption (ICH S3A & B)	Yes	Yes
Distribution (ICH S3A & B)	Yes	Yes
Metabolism (ICH S3A & B) CDER guidance: safety testing of drug metabolites	No (degradations in small peptides and single amino acids)	Yes. If metabolite represents >10% of parent compound
Excretion (ICH S3A & B)	Yes	Yes
Toxicology		
Single-dose toxicity (ICH M3(R2))	Can be helpful to select the doses for repeated-dose toxicity	Can be helpful to select the doses for repeated-dose toxicity, however no need to reach LD_{50} levels anymore
Repeated-dose toxicity (ICH M3(R2), ICH S4A)	Yes (only in relevant species)	Yes (two species, rodent and non-rodent)
Genotoxicity (ICH S2(R1))	No	Yes (in vitro and in vivo)
Carcinogenicity (ICH S1A, B, and C)	Generally, not necessary	Yes (except for anticancer agents (ICH S9)
Reproductive and developmental toxicity (ICH S5(R2))	Yes, the program could be done in a single species	Yes (two species, rodent and rabbit)
Local tolerance (ICH M3 (R2), CPMP/SWP/2145/00)	Yes, stand-alone study usually not necessary	Yes, stand-alone study usually not necessary
Immunotoxicology (ICH S8)	ICH S8 does not apply, immunogenicity and anti-drug antibodies (ADA) assessments are needed	Yes, immunotoxicity assessment
Phototoxicity (ICH S10)	No	Yes, if light absorption, generation of UV reactive species, and skin/eye distribution is achieved

Pharmacology

Pharmacodynamics

For both NCEs and biologics, the intended pharmacological target is a main factor for deciding which test systems should be selected for the nonclinical development of the drug under investigation. The Sponsor should justify the relevance of the animal species to humans taking into account the target, its structural homology, its distribution, the signal transduction pathways, and the nature of pharmacological effects. The demonstrated pharmacodynamics (PD) characteristics of a drug under development in relevant animal model(s) will be considered as the nonclinical proof of concept for NCEs as well as biologics. In contrast to the key toxicological activities (including toxicokinetics evaluation), it is acknowledged that PD studies do not need to be Good Laboratory Practice (GLP)-compliant.

Safety Pharmacology

Safety pharmacology studies should be GLP-compliant (ICH S7A&B) and need to include assessment of effects on vital functions (cardiovascular system, central nervous system (CNS), and respiratory systems) to investigate undesirable effects of a substance and its metabolites on physiological functions based on exposure at low, medium, and high doses. For some products, the evaluation of safety pharmacology endpoints can be conducted as part of toxicology and/or pharmacodynamics studies. Cardiotoxicity is a major reason why NCEs fail to reach the market. In November 2018, the establishment of Q&As for the ICH E14 and ICH S7B guidelines was endorsed. ICH S7B and ICH E14 describe nonclinical and clinical risk assessment strategies concerning the pro-arrhythmic potential of non-antiarrhythmic test substances and contribute to the design of clinical investigations. These guidelines will inform on best practices for the design, conduct, analysis, interpretation and reporting of in vitro, in silico, and in vivo nonclinical assays (as the Comprehensive in vitro Pro-arrhythmia Assessment (CiPA) initiative), in order for these assays to influence nonclinical and clinical evaluations.

Pharmacokinetics and Toxicokinetics

Guidelines ICH S3A and B require a comprehensive knowledge of the absorption, distribution, metabolism, and excretion (ADME) in view of the interpretation of pharmacology and toxicology studies. Measurement of drug concentrations (PK determinations) in biological matrices is an important aspect of medicinal product development for both NCEs and biologics. Tissue distribution studies are essential, especially in relation to potential sites of action. For NCEs, the nonclinical characterization of human metabolites is only warranted when these metabolites are observed at exposures greater than 10% of total drug-related exposure at steady-state and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies (FDA Guidance 2016, Rev 1, Safety Testing of Drug Metabolites). Such studies should be conducted to support phase 3 clinical trials. In contrast,

the expected consequence of metabolism of biologics is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood, and thus classical biotransformation studies as performed for pharmaceuticals are usually not needed.

In 2017, ICH issued a Q&A document to ICH S3A (Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies: Focus on Microsampling). This document focuses on points to consider before incorporating the microsampling method in toxicokinetics (TK) studies and acknowledges its benefits and some limitations for assessment of TK in main study animals and its overall important contribution to the 3R benefits (Replacement, Reduction, and Refinement) by reducing or eliminating the need for TK satellite animals. This recommendation is now widely in place for any pivotal studies performed.

Toxicology

As a general rule, safety evaluation programs should only include the use of relevant species. Toxicity studies in non-relevant species may be misleading and are discouraged by the regulatory authorities. A relevant species is any animal model in which the test material is pharmacologically active, and thus knowledge of receptor/epitope distribution provides a general understanding of potential in vivo toxicity of biologics. A respective "case-by-case" cross-reactivity evaluation, in vitro and/or in vivo, by immunochemical, analytical, or functional tests between species and organs/ tissues/cells should be performed to help in the selection of the relevant test system. This would optimize the evaluation of toxicity arising from the binding to the receptor/epitope and any unintentional tissue cross-reactivity. An animal species which does not express the desired receptor/epitope may still be of some relevance for assessing toxicity if comparable unintentional tissue cross-reactivity to humans is demonstrated.

Repeated-Dose Toxicity

For NCEs, repeated-dose toxicity studies in two species are normally required. The studies should be designed to reflect the intended clinical use (duration and frequency of administration, clinical route of administration) and take into account the therapeutic indication. Frequency of administration is based on PD, PK, and toxicological profile. Dose levels often include a low (pharmacological), an intermediate, and a high (potentially toxic) dose. Of note, the regulation recommends multiple approaches for the highest dose of general, i.e., repeated-dose toxicity studies). When possible, this includes a maximum dose 50-fold the therapeutic target. A control group should also always be included.

For biologics, however, it is not rare to note that the pivotal toxicity program can be performed in a single species only (i.e., cynomolgus monkey). In case no relevant species can be determined, the use of transgenic animals expressing the human receptor or the use of homologous proteins should be considered. The information gained from the use of a transgenic animal model expressing the human receptor is

optimized when the interaction of the product and the humanized receptor has similar physiological consequences to those expected in humans.

ICH M3(R2) requires treatment durations in non-rodents (9 months) and rodents (6 months) to enable long-term administration to humans (>6 months). It is noteworthy that for non-rodent animals, a treatment for 6 months may usually be sufficient in the EU, yet to ensure a global approval, it is nevertheless preferable to treat non-rodents animals for 9 months.

For both NCEs and biologics, the evaluation of local tolerance (ICH M3(R2)) by the intended clinical route of administration is performed as part of the general toxicity studies. Stand-alone studies are generally not recommended.

Genotoxicity and Carcinogenicity

Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect compounds that induce genetic damage by various mechanisms, such as gene mutations, chromosomal damage, or recombination. Extensive reviews have shown that many NCEs that are mutagenic in the bacterial reverse mutation (i.e., AMES) test are rodent carcinogens. To increase the sensitivity for detection of carcinogens, a battery approach has been implemented because no single test is capable of detecting all genotoxic mechanisms relevant in tumorigenesis. Thus, ICH S2(R1) states that if an assay for gene mutation is sufficient to support single-dose clinical trials with NCEs, a complete battery of genotoxicity tests, such as an AMES test, a cytogenetic test for chromosomal damage, and in vivo test for genotoxicity, should be completed before initiation of phase 2 trials, as well as to later on support the marketing of a product. Of note, genotoxicity studies are not considered essential to support clinical trials for therapeutics intended to treat patients with late stage or advanced cancer.

ICH S6(R1) states that biopharmaceuticals do not need to be tested for genotoxicity, as standard proteins and peptides are not supposed to induce damages at the DNA/chromosomal level.

Moreover, ICH M7(R1) has been introduced to define the maximal levels of daily mutagenic impurities which can be authorized within a NCE formulation. Importantly, genotoxicity studies are not applicable to biologics and therefore are presently not required (see ICH S6(R1)).

Conditions relevant for the carcinogenicity testing of NCEs are discussed in ICH S1A. In general, carcinogenicity studies should be conducted to support the marketing application and thus logically launched during phase 3. However, for pharmaceuticals developed to treat certain serious diseases, in order to speed up the development process, it is possible to discuss the timing with the agencies and conduct carcinogenicity studies post-approval. The basic scheme is one long-term rodent carcinogenicity study and one other study both supported with TK data (e.g., in vivo tests). A change to the current S1 guideline is foreseen and expected to introduce a more comprehensive and integrated approach to address the risk for human carcinogenicity. Ideally, this analytical approach will yield sufficiently instructive criteria for when a work-of-evidence option would be preferable to a 2-year bioassay in a development program, thereby improving assessment of human

carcinogenic risk of pharmaceuticals and minimizing regulatory discordance across regions. As mentioned in ICH S6(R1), standard carcinogenicity bioassays are generally inappropriate for biologics. However, product-specific assessment of carcinogenic potential may still be needed depending upon the duration of clinical dosing, patient population, and/or biological activity of the product (e.g., growth factors, immunosuppressive agents).

Reprotoxicity and Juvenile Animal Studies

As a consequence of the thalidomide disaster in the early 1960s (Vargesson 2015), reproduction toxicity studies should be conducted as appropriate for the population to be exposed (ICH S5(R2)). The goal is to reveal any adverse effect of the product on mammalian reproduction. The combination of studies (fertility, embryo-fetal, and peri-/postnatal assessment) selected should cover all stages of development from conception to sexual maturity. For NCEs, two species should be tested (rats and rabbits) to assess the potential of a NCE on the embryo/fetal development, as the thalidomide disaster revealed that mice are less sensitive to thalidomide than other species such as rabbits (Vargesson 2015). Observation should be performed from conception in one generation through conception in the following generation (complete life cycle).

For biologics, reproductive toxicity studies should also be conducted in accordance with the principles outlined in ICH S5(R2). However, one species only can be sufficient to address effects on embryo-fetal development. This guideline is currently under revision since 2015 (ICH S5(R3) EWG Revision of S5 Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals). Interestingly, this draft version mentions the notion of Weight of Evidence (WoE). This is based on the fact that toxicity studies in pregnant animals are not always necessary for assessing the human risk of developmental toxicity of biopharmaceuticals. Therefore, the accumulated knowledge on target biology and molecule-specific pharmacokinetics should allow to accurately anticipate the effects of target activation by biopharmaceuticals using a WoE approach (Rocca et al. 2018). Such a WoE-based assessment should include all available data including target biology, pharmacokinetics, class effects, genetically modified animals, human mutations, and an exhaustive literature review. Such an evaluation may be sufficient to inform risk for specific clinical indications and patient populations, even though this approach is currently only applicable for oncology drugs and biologics. Noteworthy as well is that ICH S9 states to support the treatment of patients with late-stage or advanced cancer, generally neither warrants a fertility study nor any peri- and postnatal toxicology study. These are clear examples for modernizing testing paradigms to enhance human risk assessment while also potentially reducing animal use, notwithstanding that there are further areas where the guideline could be revised or amended for greater clarity, as well as to align more fully with other more recent ICH guidelines such as ICH M3(R2), ICH S6 (R1) as well as ICH S9.

As regards juvenile animal studies (JAS), a draft 2 guideline (ICH S11) was issued in 2018 to ultimately harmonize regional guidelines from various agencies to reach an agreement on the need for, timing of, and design of JAS and thus allow a

common development program of medicines for the pediatric population. This is particularly relevant in the EU, where filing for a marketing authorization, even for an indication intended for adults only, will be systematically refused by the EMA in the absence of a Pediatric Investigation Plan (PIP) previously accepted by the Pediatric Committee of the agency.

Immunogenicity and Immunotoxicity

In contrast to the majority of NCEs, many biologics intended for human are immunogenic in animals. The immunogenicity of biologics can cause hypersensitivity responses, anaphylaxis, and infusion reactions (Rosenberg 2003). Anti-drug antibody (ADA) responses could affect the efficacy and/or safety of protein therapeutics and/or complicate interpretation of the toxicity, pharmacokinetic, and pharmacodynamic data. It is also known that particular glycosylation patterns might be immunogenic and some protein aggregates might trigger immunogenicity. Animal models are increasingly used to study immunogenicity of therapeutic proteins. They are employed as predictive tools to assess immunogenicity during drug development and have become vital in studying the mechanisms underlying immunogenicity of therapeutic proteins. However, the use of animal models needs critical evaluation (Brinks 2011). Because of species differences, the predictive value of these animal models is limited.

It is widely acknowledged that biologics often reveal their real immunotoxicity potential for humans only during clinical studies. The predictive value of animal studies and traditional in vitro screens is thus questionable. Despite these limitations, antibody levels associated with administration of biologics should be measured during repeated-dose toxicity studies. Antibody responses should be characterized (titer, number of responding animals, neutralizing or non-neutralizing), and their appearance should be correlated with any pharmacological and/or toxicological changes. Specifically, the effects of antibody formation on PK/PD parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data. Possible pathological changes related to immune complex formation and deposition should be evaluated.

In line with the above comments, both the FDA and the EMA recently issued guidelines on these topics. The FDA released a guidance on the development of biologics and biosimilars (FDA Guidance for Industry 2019). The FDA recommends a multi-tiered testing approach, and the document spells out the development and validation of screening, confirmatory, titration, as well as neutralization assays. In the EU, the guideline on the immunogenicity assessment of therapeutic proteins (EMEA/CHMP/BMWP/14327/2006 Rev 1) states clearly that the current predictive value of animal studies for evaluation of immunogenicity of a biological medicinal product in humans is low due to differences between human and animal immune systems and to immunogenicity of human proteins in animals. The development of adequate screening and confirmatory ADA assays to measure immune responses against a therapeutic protein is the basis of the evaluation of immunogenicity.

As regards NCEs, dedicated immunotoxicity studies are mandatory only when a cause for concern is identified in the repeated-dose toxicity studies (ICH S8). In this case, additional immunotoxicity studies should be performed to verify the immunotoxic potential of the compound, completed before exposure of a large population (before phase 3).

The Standard for Exchange of Nonclinical Data (SEND) Format

As regards the submission of toxicology and safety reports to regulatory agencies, the SEND initiative has been recently implemented by the FDA (Demollari 2019; Carfagna et al. 2020) in order to submit nonclinical data in a structured manner. The format was created by the Clinical Data Interchange Standards Consortium (CDISC). The governing document of the SEND standard is the Standard for Exchange of Nonclinical Data Implementation Guide (SENDIG). It describes the rules for providing standardized data according to the study data tabulation model (SDTM) for nonclinical studies.

The FDA requests that all nonclinical data from safety studies started on or after December 17, 2016, should be presented according to SEND. This applies to New Drug Applications (NDAs), Biologic License Applications (BLAs), and Abbreviated New Drug Applications (ANDAs), and for Investigational New Drugs (INDs) after December 17, 2017. Our strong advice to Sponsors is to ensure that all single/repeated-dose toxicity, carcinogenicity, and safety pharmacology (on cardiovascular and respiratory evaluation) data are provided in adequate format. The modalities should be discussed and aligned in-house or with the selected Contract Research Organization where the study will be performed.

Impact of Manufacturing and Formulation Changes on the Development Process

The performance of safety bridging strategies within batches of the same biological produced at different scales is a key element to master in order to obtain clinical trial and marketing authorization. The use of cells of human, animal, or even plant origin for the production of biologics is subject to potential contamination. A change in manufacturing process and/or of formulation of the product represents a potential risk for patients (such as immune-suppression, immuno-stimulation, hypersensitivity, and autoimmunity). Particular attention must be paid to the characterization, purity, and stability of the starting materials, as well as the presence of aggregates. Products should be tested for viral safety (ICH Q5A(R1) Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin) and genetic stability (ICH Q5B Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines Used for Production of r-DNA Derived Protein Products). A European guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials was adopted in 2008 and revised in 2018 (EMA/CHMP/BWP/534898/2008 rev.1 corrigendum).

The production process must provide relatively large amounts of test material. The degree of comparability of the test material from batch to batch in the

development program requires an early validation of the production and testing methods as well as the precise definition of the product specifications (EMEA/CHMP/BMWP/101695/2006). An early well-designed bridging strategy in terms of upscale process is preferable to a subsequent full test program.

Alternative Methods Including In Silico Evaluation

Animal models are widely used for a long time for toxicity testing. However, in vivo animal tests are constrained by time, ethical considerations, and financial burden. ICH M3(R2) states that "consideration should be given to use of new in vitro alternative methods for safety evaluation. These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard methods." Therefore, not surprisingly, this last decade has seen the emergence of a paradigm shift: in line with the 3Rs principle (reduce/refine/replace), regulatory agencies now appear more open to alternative approaches to decrease the number of animals during the nonclinical development of NCEs and biologicals. This change is particularly apparent in the draft guidance ICH S5(R3), which once adopted may take into consideration data from, e.g., qualified alternative in vitro and non-mammalian assays, in combination with one or more in vivo mammalian embryo-fetal development studies. For tolerance assessment, alternative methods on 3D reconstructed human epithelium (ocular or cutaneous) can already replace the previous eye and skin irritation studies in rabbit.

In silico toxicology is one type of toxicity assessment that uses computational methods to analyze, simulate, visualize, or predict the toxicity of chemicals. In silico toxicology aims to complement existing toxicity tests to predict toxicity, prioritize chemicals, guide toxicity tests, and minimize late-stage failures in drug design (Raies and Bajic 2016). In silico toxicology methods involve a wide variety of computational tools: databases for storing data about chemicals, their toxicity, and chemical properties; software for generating molecular descriptors; simulation tools for systems biology and molecular dynamics; modeling methods for toxicity prediction; modeling tools such as statistical packages and software for generating prediction models; expert systems that include pre-built models in web servers or standalone applications for predicting toxicity; and visualization tools. A plethora of databases (almost 1000) exist online for in silico drug safety assessment. A recent review (Pawar et al. 2019) provides a comprehensive listing of the key in silico data resources such as chemical identity and properties, mechanism of action, toxicology, exposure, ADME properties, clinical trials, pharmacovigilance, patent-related databases, protein-protein interactions, and, finally, databases related to animal alternatives in support of 3Rs policies.

Finally, based on our own experience, the FDA seems more advanced than the EMA as regards modeling and simulation (M&S), having already identified M&S tools as one of the priorities to improve in the FDA's 2011 Strategic Plan for Advancing Regulatory Science. This plan included the need for developing also clinical trial simulation models that can reveal interactions between drug or device

effects, patient characteristics, and disease variables influencing outcomes, as well as development of data management tools to inform computer model development, clinical risk prediction, and regulatory decision-making (Rousseau et al. 2019).

Environmental Risk Assessment (ERA)

An Environmental Risk Assessment is required by EMA since 2006 for all new marketing authorization applications (MAA) for a medicinal product through a centralized, mutual recognition and decentralized and national procedure regardless of its legal basis. This affects all new products (with some exceptions like vitamins, amino acids, peptides, electrolytes, and herbal products), including already marketed drugs, if, e.g., a new indication results in significant increase in their extent of use (EMEA/CHMP/SWP/4447/00 corr 2, 2006). A revised draft guideline is in preparation with a consultation period closed in June 2019 (EMEA/CHMP/SWP/4447/00 Rev 1, 2018). These guidelines follow a risk-based approach based on environmental release of the pharmaceutical, with testing dictated by partitioning; solubility; persistent, bioaccumulative, and toxic (PBT) characteristics; and endocrine activity.

Even if the ERA requirements in the USA may appear less stringent than in the EU, the FDA stipulates also that a risk assessment or categorical exclusion claim should accompany every IND, NDA, or BLA (FDA/CDER/CBER/1998). In 2016, FDA supplemented this guidance (FDA/CDER/2016) by addressing specific considerations for drugs that have potential estrogenic, androgenic, or thyroid hormone pathway activity in the environment.

Conclusion and Recommendations

Biologicals can provide more innovative, effective, and targeted therapies for numerous diseases than NCEs. In order to detect any potential toxicity of these promising products, the determination of the safe dosage at the start of clinical studies and the establishment of dose–response relationships, a rationale "case-by-case" nonclinical testing strategy, should be put in place taking into account not only ICH S6(R1) (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) but also all other guidelines listed in this chapter. Biologicals differ in many aspects from the more conventional NCE drugs, because of their species- and tissue-specific characteristics and their immunogenicity potential due to their particular nature and complex mode of production.

With biologicals, the required safety testing may appear more science-driven and designed around the product and the predicted safety issues resulting from the biology (mechanism of action), rather than being a standard list of tests. This is because toxicity with biologicals is, in the vast majority of cases, secondary to the pharmacology, whereas NCEs can have off-target effects unrelated to the biology or pharmacology.

It is also our view that, in the next decade(s), the importance of alternative methods (in vitro and in silico) to assess the toxicity of our future medicines will considerably expand. Such a (r)evolution is already initiated and may not only impact animal research. Indeed, the FDA initiated a pilot program known as the model-informed drug development (MIDD) in which the agency meets with drug developers to discuss and agree on which key program decisions can be supported by mathematical models and simulations. The FDA may even accept in the near future some selected clinical trials performed with virtual patients.

Finally, in order to avoid critical issues at the time of marketing authorization application, we strongly advise any drug developer to request a timely scientific advice meeting with a regulatory agency to discuss and find an agreement on the relevance of the nonclinical development program they intend to perform.

Cross-References

► Microbiome Product Toxicology: Regulatory View on Translational Challenges
► Toxicological Aspects in the Regulation of Gene Therapy

Disclaimer Any views and opinions expressed in this article chapter are those of the authors and do not necessarily reflect those of any institutions the authors are associated with.

References

Peer-Reviewed Articles

Baumann A (2009) Nonclinical development of biopharmaceuticals. Drug Discov Today 14(23–24):1112–1122

Brinks V (2011) Immunogenicity of therapeutic proteins: the use of animal models. Pharm Res 28:2379–2385

Carfagna MA, Bjerregaard TG, Fukushima T, Houser W, Sloan C, Snyder K, Anderson J, Page T (2020) SEND harmonization & cross-study analysis: a proposal to better harvest the value from SEND data. Regul Toxicol Pharmacol 111:104542

Demollari M (2019) Moving from unstructured to structured data – US nonclinical data standards. Regulatory Rapporteur 16:9–12

Pawar G, Madden JC, Ebbrell D, Firman JW, Cronin MTD (2019) In silico toxicology data resources to support read-across and (Q)SAR. Front Pharmacol 10:1–25

Raies AB, Bajic VB (2016) *In silico* toxicology: computational methods for the prediction of chemical toxicity. WIREs Comput Mol Sci 6:147–172

Rocca M, Morford LL, Blanset DL, Halpern WG, Cavagnaro J, Bowman CJ (2018) Applying a weight of evidence approach to the evaluation of developmental toxicity of biopharmaceuticals. Regul Toxicol Pharmacol 98:69–79

Rosenberg AS (2003) Immunogenicity of biological therapeutics: a hierarchy of concerns. Dev Biol (Basel) 112:15–21

Rousseau C, Crozatier C, duVerle DA, Buyse M, Carter SE, Voisin EM, Boissel FH (2019) A regulatory landscape shift for *in silico* clinical trials. Regulatory Rapporteur 16:18–21

Schwieterman WD (2006) Regulating biopharmaceuticals under CDER versus CBER: an insider's perspective. Drug Discov Today 19–20:945–951
Singh H (2018) French phase I clinical trial disaster: issues, learning points and potential safety measures. J Nat Sci Biol Med 9:106–110
Suntharalingam G (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. New Engl J Med 355(10):1018–1028
Vargesson N (2015) Review thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C 105:140–156

Regulatory Documents

EMA/CHMP/BWP/534898/2008 rev. 1 corrigendum (2018): Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials
EMA/CHMP/SWP/44609/2010 rev 1: Question and answers on 'Guideline on the environmental risk assessment of medicinal products for human use
EMEA/CHMP/BMWP/101695/2006: Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process non-clinical and clinical issues
EMEA/CHMP/EWP/192217/2009: Guideline on bioanalytical method validation
EMEA/CHMP/SWP/28367/07 Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products
EMEA/CHMP/SWP/28367/07(R1)/2017: Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products
EMEA/CHMP/SWP/4447/00 corr 2/2006: Guideline on the environmental risk assessment of medicinal products for human use
EMEA/CHMP/SWP/4447/00 Rev 1/draft/2018: Guideline on the environmental risk assessment of medicinal products for human use
FDA Guidance for Industry (1998) Environmental assessment of human drug and biologics applications
FDA Guidance for Industry (2005) Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers
FDA Guidance for Industry (2008) Safety testing of drug metabolites
FDA Guidance for Industry (2016) Environmental assessment: question and answers regarding drugs with estrogenic, androgenic or thyroid activity
FDA Guidance for Industry (2019) Immunogenicity testing of therapeutic protein products – developing and validating assays for anti-drug antibody detection. Can be downloaded from https://www.fda.gov/media/119788/download
FDA Guidance: all the FDA Guidance listed in this document and their corresponding associated files can be freely downloaded on the https://www.fda.gov/regulatory-information/search-fda-guidance-documents website
ICH guidelines: all the ICH guidelines listed in this document and their corresponding associated files can be freely downloaded on the https://www.ich.org website
ICH Status Report (2019). The ICHS1 regulatory testing paradigm of carcinogenicity in rats – status report 019. Safety Guidelines https://database.ich.org/sites/default/files/S1_StatusReport_2019_0802.pdf
Notice to Applicants Volume 2A (2016) Procedures for marketing authorization. Can be downloaded from https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-2/vol2a_chap1_rev6_201612.pdf