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Workshop Report

New challenges and opportunities in nonclinical safety testing of biologics ☆

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ABSTRACT

New challenges and opportunities in nonclinical safety testing of biologics were discussed at the 3rd European BioSafe Annual General Membership meeting in November 2013 in Berlin:

(i) Approaches to refine use of non-human primates in non-clinical safety testing of biologics and current experience on the use of minipigs as alternative non-rodent species.
(ii) Tissue distribution studies as a useful tool to support pharmacokinetic/pharmacodynamic (PKPD) assessment of biologics, in that they provide valuable mechanistic insights at drug levels at the site of action.
(iii) Mechanisms of nonspecific toxicity of antibody drug conjugates (ADC) and ways to increase the safety margins.
(iv) Although biologics toxicity typically manifests as exaggerated pharmacology there are some reported case studies on unexpected toxicity.
(v) Specifics of non-clinical development approaches of noncanonical monoclonal antibodies (mAbs), like bispecifics and nanobodies.

1. Introduction

BioSafe is the Preclinical Safety expert group of the Biotechnology Industry Organization (BIO), which has been tasked with the mission to serve as a resource for BIO members and BIO staff by identifying and responding to key scientific and regulatory issues related to the preclinical safety evaluation of biopharmaceutical products. Beyond its general membership meetings in the U.S., BioSafe has started to run in parallel yearly European Meetings to foster face to face discussions with European colleagues of BIO member companies.

The 3rd Annual BioSafe European General Membership meeting was hosted by Bayer Pharma on November 18–19, 2013 in Berlin. The 80 scientists (65 from Europe, 15 from U.S. and Japan) – with toxicology, pathology or pharmacokinetic background – represented global big pharmaceutical/biotechnology companies, small biotechnology companies and individual contract research organizations. At this year's meeting new challenges in non-clinical development of biologics were discussed, including animal use and species selection, unexpected toxicities, distribution behavior and specifics of antibody drug conjugate and non-traditional mAb development. At each session, case examples were presented followed by podium discussions.

☆ New challenges and opportunities in nonclinical safety testing of biologics were discussed at the 3rd European BioSafe Annual General Membership meeting in November 2013, addressing scientific, strategic and experimental approaches in Toxicology and Pharmacokinetics.

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2. Animal use in biologics development

With the increasing importance of biologics in drug development, non-human primates (NHP) have been identified as the most suitable and relevant toxicology species leading to a higher demand of this species for non-clinical safety testing of biologics. Beyond the increasing ethical and public pressure to explore and advance approaches to reduce the number of NHPs (Bluemel, 2012), it was recently questioned why NHPs are used in biologics development, when pharmacology-mediated adverse effects of monoclonal antibodies (mAbs) are highly predictive from in vitro studies (Van Meer et al., 2013). In the introduction of this session chaired by Jenny Sims (Integrated Biologix) and Andreas Baumann (Bayer Pharma), it was questioned if this statement is only a future dream or if it has some realistic components. If pharmacology-mediated adverse effects and PKPD relationships are understood with short-term animal studies, what is gained from further chronic toxicity studies. The following two lectures reviewed approaches to refine NHP use in non-clinical safety testing in biologics development without compromising the risk benefit assessments for human use.

Kathryn Chapman (U.K. National Centre for 3R's, NC3Rs) presented approaches to minimize the use of NHPs in biologics development. There has been particular interest in animal use in biologic testing since it was recognized that the NHP may be the only relevant non-clinical toxicology species for many of these products. The NC3Rs, in collaboration with up to 30 organizations from the pharmaceutical, biotechnology, contract research and regulatory environment, have facilitated cross-company data-sharing initiatives to minimize the increase in NHP use (Chapman et al., 0000). These evidence-based approaches have fed into regulatory addendums e.g., ICH S6 (R1) and ICH M3 (R2) and continue to support the field in using appropriate study designs to answer the scientific questions at hand. Two current hot topics in this area with a focus on the 3Rs (replacement, refinement and reduction of animals in research) are (i) how often rodent models can support biologic development and (ii) how and when recovery animals should be included on studies. Unpublished data shows that company portfolios for mAbs range from having no products with rodent potency to a third of their pipeline having the potential to use the rodents for some studies. This is linked to therapeutic area, for example less frequent rodent potency for immunology products. Also the company strategy in screening for rodent potency in candidate selection and development varies depending on therapeutic area. There are case studies showing that rodent models do support biologic programs and have the potential to provide more relevant data and reduce the use of NHP on some occasions. With the revision of ICH S6 (R1) Guideline (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals), which describes the potential for only using the rodent in (sub) chronic studies if the toxicity profile of the rodent and NHP is the same in short term studies, the prediction is that the rodent will be used more for development of these products. In addition, technological advances such as microsampling mean that rodent data is likely to be used more frequently to support clinical trials.

The use of recovery animals has been identified as another area where animal use is increasing. Often recovery animals are included on all studies conducted and more than one dose group. The question is whether the reasons behind this are scientifically driven or whether it is upward creep. A cross-company data sharing group has looked at 259 studies from 137 compounds and 22 companies and these data show wide variation in the number of recovery animals used. Analysis shows that there are opportunities to reduce the use of recovery animals in certain circumstances which would not impact drug development.

In addition to the in vivo approaches there is also ongoing work to identify the benefits and limitations of in vitro technologies to assess the safety profile of biologics. A holistic, integrated approach to get the best data from the most appropriate technology or species is a 'must have' in the future of biologics products.

Lauren Black (Charles River Laboratories) gave some further insights on the use of satellite groups and blood sample volume reduction. For many years different animals were used for toxicity evaluation (satellite animals) than those assayed for blood levels (toxicokinetic = TK groups). Assays of the TK were done in satellite groups because the analytical methods were not sensitive and required up to 500 µl of blood to be drawn for each sample. Such high blood volumes would deplete the rodent's hematocrit if drawn multiple times from the same animal, and this would confound interpretation of toxicity if performed in the "main study" animals designated for pathology. So, until very recently, the rodent numbers used for satellite TK and or pharmacodynamics (PD) could end up being half of the animals utilized on the study, and total number is often high (~300). The satellite animals were not used for any other endpoints, other than TK or PD (no pathology and no intercurrent clinical pathology). In contrast to rodent studies, large animal experiments are conducted much more translationally, where self baselines are routinely available. In these cases, far fewer large animals are used (~30).

It would be optimal to gain all data from each (rodent) animal utilized in toxicology studies, and correlate a given animal's toxicity and PD measures, with its own TK. The only way to achieve more insight from each animal, depends on two advances; first – refined, methods for taking repeated blood draws from the rodent; and second – developing assay methods that utilize far smaller samples of blood (Powles-Glover et al., 2014).

This way, dynamic insights into animals TK, PD, and clinical pathology effects might be gained, without undue stress to the animal, or confounding toxic effects from repeated blood draws. Such advances have been developed in many labs using capillary based microsampling of only 32 µl of blood, generally referred to as microsampling. Micro-ELISA methods have also been developed, and have not posed severe technical hurdles; typically, serum from biologic drug-treated animals must be diluted anyway, to enable assays to fall within standard curves.

With improved insight, interpretation, and translation, more insightful toxicity studies may be designed in rodents, which may alleviate some need for NHP work.

3. Use of minipigs in non-clinical safety testing with biologics – Quo vadis ?

Minipigs are increasingly used as non-rodent species for toxicity testing of pharmaceuticals, in particular in Europe (Svendsen, 2006; Ganderup et al., 2012). However, the focus is largely on small molecule-based therapeutics and dermal administration (Ganderup, 2011); only few data exist on repeat-dose IV administration of biologics. The session chaired by Sven Kronenberg (Hoffmann-La Roche) and Roy Forster (CiToxLAB) provided an overview about the use of minipigs in safety testing of biologics. A gap analysis on the use of mAbs in the minipig by Kronenberg emphasized that the minipig immune system has a largely analogous structure and function to the human immune system (Bode et al., 2010), but a better understanding on how sensitive minipigs are towards infusion-related reactions and FcγR-mediated effector function is yet missing. This includes possible (side) effects of IV administration of mAbs, such as cytokine release, complement activation and ADCC. Some of the effects can be caused also by polymer excipients used in biologics formulations: minipigs, similar to dogs, show acute cardio-pulmonary reactions to some

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