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Toxicity Testing in the 21st Century: Challenges and Perspectives

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Editorial

Nonclinical toxicity testing of chemicals and chemically-derived small molecule pharmaceuticals as well as biotechnology-derived large molecule pharmaceuticals plays a critical role in risk assessment and risk mitigation. While toxicity testing of chemicals aims to identify and manage hazards to human health and the environment following intended or accidental exposure, pharmaceutical toxicity testing forms a critical part of the risk-benefit assessment which balances the intended beneficial effects on the patient health against observed toxicities and potential adverse effects. The importance of nonclinical toxicity testing and adequate data interpretation in ensuring human safety is underscored by the thalidomide calamity in the early 1960's where world-wide approximately 10,000 children were born with limb malformations [1-3]. A more recent incident in 2006 involved TGN1412 [4,5]. Administration of TGN1412, a super-agonistic monoclonal antibody against the T-cell co-stimulatory molecule CD28 resulted in a severe, uncontrolled cytokine release syndrome and life-threatening multi-organ failure in 6 volunteers in a first time in human clinical trial. Although both incidents happened more than 4 decades apart from each other, the triggering event in both cases can be broadly attributed to the failure to adequately mirror the human toxicity with the nonclinical test systems used at the time. The growing insight in pathophysiological processes, the constantly evolving landscape of molecular targets for therapeutic intervention and the dramatically increasing heterogeneity of new molecular entities require a continuous adaptation and refinement of the guiding principles and practices of nonclinical toxicology assessments during drug development. In addition, the 2007 implementation of the REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemical Substances) [6] in Europe has an impact on the established practice for toxicity testing of chemicals. The large number of marketed chemicals with limited or even absent toxicology data, which still must be tested and registered at the European Chemicals Agency, require alternative approaches beyond the classical *in vivo* testing in various animal species.

Notably, while the primary goal of the nonclinical toxicity assessment as outlined above has not changed over the past decades, there is a considerable change in the practice of toxicology assessments and also an increasing demand for nonclinical safety data during early drug development. In order to adapt to this changing environment, various challenges for toxicity testing in the 21st century need to be adequately addressed. These challenges include the following: (1) the demand for predictive screening assays to assess safety endpoints early in pharmaceutical drug development to either reduce the attrition rate due to toxicity or to support a rational structure-based drug design; (2) the need for medium to high-throughput approaches to enable the parallel assessment of multiple compounds; (3) the adaptation of cutting-edge technologies such as microarray techniques and *in vivo* imaging techniques like magnetic resonance imaging (MRI) or positron emission tomography (PET) in toxicology study protocols; (4) the identification of alternatives to the classical *in vivo* toxicology study paradigm using rodent and/or non-rodent test species.

The current industry practice for the development of chemical-derived new molecular pharmaceutical entities involve an extensive early screening process in order to rank various candidates and select the most promising candidate based on defined chemical and biological attributes. This approach is designed to mitigate certain development risks and reduce the attrition rate in later stages of drug development. The value of *in vitro* assays to guide the early lead selection process by screening for various pharmacological properties and initial metabolic characteristics is well established. Likewise, *in vitro* genotoxicity screenings, where a number of different assays like the miniaturized Ames assay [7] or the so-called GreenScreen HC assay [8], are well established components of early lead selection funnels. In contrast, there is an ongoing controversial debate about the predicivity and usefulness of early screening assays addressing more complex toxicological endpoints like reproductive toxicity, systemic toxicities such as hepato-/cardiotoxicity, or even idiosyncratic toxicities. Although some progress has been made in developing *in vitro* methods to screen for developmental toxicity [9,10], for example, there is still a need for additional methods suitable for use within the early lead selection campaigns. Improving the predictive capability of *in vitro* toxicity assays in these areas would not only enhance the accuracy of early lead selection processes, but would also benefit efforts to replace animal testing.

In addition, the increasing demand for toxicology data prior to selection of a lead candidate requires the development of new, medium- or high throughput testing protocols. The traditional *in vivo* approach for toxicity testing is not feasible for a parallel analysis of several development candidates. While most of the traditional toxicology testing protocols is tailored to fulfill the requirements of the various regulatory authorities, a screening assay approach would require a different way of thinking that must balance technical feasibility, specificity, sensitivity and throughput in order to enable a ranking of different compounds based on their safety attributes. *In vitro* methods that allow a high throughput, fast data turnaround time, and minimal amounts of test substances are desired in order to accommodate the special needs of early predictive safety screening strategies.

Another area of increasing importance for future toxicology testing strategies is the implementation of the principles of 3R (Reduce, Refine and Replace) [11] in toxicology study design. Both the increasing public criticism and pressure against research involving animals, especially on studies involving non-human primates [12], and the inclusion of the 3R principles in recent regulatory guidance updates, call for attempts to reduce the number of animals used for nonclinical toxicology

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studies. Recent efforts have been focused on the critical analysis and subsequent optimization of non-human primate study designs because the demand for toxicity testing in nonhuman primates, mostly in Cynomologus monkeys, is increasing due to the heavily growing share of large molecule drugs in the biopharmaceutical industry pipeline. Due to the often exclusive species specificity of monoclonal antibodies, the nonhuman primate is in many cases the only relevant species for nonclinical safety assessment. Some areas for optimization have been identified [13,14] and some practical measures such as the inclusion of safety pharmacology endpoints in toxicology studies or the implementation of the so-called enhanced Pre- and Postnatal Development (ePPND) study design [15,16] in regulatory guidance documents, are already in place. Both approaches eliminate the need for stand-alone safety pharmacology or embryo-fetal development studies and thus reduce the number of animals used. Another controversial topic that has been discussed and will gain more and more momentum in future years is the complete replacement of animal-based studies in toxicology testing. Although some progress in the field has already been achieved and several methods have been validated and are also used for regulatory toxicity testing of chemicals [17], a complete replacement of animals in toxicology studies is scientifically not justified. Regardless, the huge demand for basic toxicology data on a vast amount of marketed chemicals to comply with the REACH regulation may draw even more attention to partial replacement strategies.

The primary goal of a nonclinical toxicology study is to identify potential target organs of toxicity, assess the reversibility of observed findings and to establish a dose-response relationship. However, the emerging area that facilitates the translation of animal findings into man and the bridging between preclinical and clinical science, often require data beyond the typical analyses that are traditionally included in a standard toxicology study protocol. New and emerging techniques like microarray techniques for pharmaco-/toxicogenomic analyses, predictive safety biomarker analyses using flow cytometry or *in vivo* imaging techniques like magnetic resonance imaging (MRI), computed tomography (CT), or positron emission tomography (PET) are becoming of increasingly important for nonclinical studies. Hereby, the focus is on techniques or read-outs that can directly be translated from the animal study into a human clinical trial.

Historically, nonclinical toxicity testing of pharmaceutical drug development candidates and industrial chemicals was a relatively standardized and guideline-driven process which relied almost exclusively on various *in vivo* studies in rodent and non-rodent animal species. However, combining the increasing demand for early safety assessments, progress in experimental methodology, and the increasing public pressure to ban animal experiments makes it imperative to develop new and alternative approaches for toxicity testing in the 21st century. These approaches need to include more flexible case by case strategies that incorporate emerging techniques of clinical relevance and the use of alternative *in vitro* or in silico methods that do not compromise human risk assessment or risk mitigation.

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