METHODS IN MOLECULAR BIOLOGY

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Drug Safety Evaluation

Methods and Protocols

Second Edition

Edited by

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Preface

Nonclinical drug safety evaluation is the assessment of the safety profile of therapeutic agents through the conduct of laboratory studies in in vitro systems and in animals. The main objectives of drug safety evaluation studies are to differentiate between new drug entities that are unacceptably toxic and those that are not, characterize the potential adverse effects of new drugs, determine animal dosage levels that do not cause toxicity, and estimate safe dosages to be used in clinical studies. Several types of studies are conducted in drug safety evaluation: acute to chronic general toxicity studies, reproductive toxicity studies, genotoxicity studies, carcinogenicity studies, safety pharmacology studies, and investigative toxicity studies.

The volume *Drug Safety Evaluation Methods and Protocols* was published as an earlier edition in the *Methods in Molecular Biology* series in 2011. In this second edition, some of the chapters from the previous edition have been updated to take into account the most recent advances in this field. In addition, several new chapters have been added to describe innovative tools and methods that have been developed and applied in the last 6 years to improve the safety evaluation of new drug candidates. Part I presents specific aspects related to the experimental design of toxicity studies conducted to support combination drugs in humans and pediatric indications. Part II focuses on Pathology with two chapters on detailed procedures of necropsy, tissue sampling, and processing for histopathological evaluation, and one chapter on the principles and methods of immunohistochemistry. A new chapter describes the applications of mass spectrometry imaging which are gaining more and more interest to detect and map compounds within tissue sections, thus providing useful information on drug distribution in relation to histopathological findings. Part III provides detailed protocols on Genetic Toxicology. A chapter describes the comet assay, a sensitive electrophoretic method for measuring DNA strand breaks at the level of single cells, together with the use of bacterial repair endonucleases to detect specific DNA lesions. Two new chapters describe recent advancements of the *Pig-a* mutation assay, which is anticipated to be a useful trans-species tool for evaluating the in vivo mutagenicity of chemicals and has been proposed as a new tool for preclinical safety assessment of regulated chemicals. Part IV provides methods on Safety Pharmacology, in which progress has been made to early evaluate the cardiosafety liability of new drug candidates. A new chapter describes how automatization procedures can be used to replace the resources and time-consuming manual patch-clamp approach for the hERG cardiac potassium channel. Another new chapter describes a method for measuring the contractility of induced human pluripotent stem cell derived cardiomyocytes using an impedance measurement system. Part V details methods on Investigative Toxicology aimed at better understanding mechanisms of toxicity and assessing potential translation to humans. Several chapters from the first edition have been updated on protocols and applications of nuclear magnetic resonance and mass spectrometry methods for metabolomics in several biological matrices, statistical analysis of real-time reverse transcription polymerase chain reaction (RT-PCR) which is a benchmark technology to detect and quantify mRNAs, and in vitro evaluation of mitochondrial respiration in cultured rat hepatocytes. A new chapter has been added on the early in silico evaluation of target safety, an approach which has now proven to be an invaluable resource

to support both drug discovery and development phases. Part VI is dedicated to screening assays for Developmental Toxicity. Two chapters provide detailed protocols of the Fetax assay and the Zebrafish model. Part VII encompasses methods to identify and characterize novel translational safety biomarkers to better monitor potential toxicity of drug candidates in both preclinical and clinical studies. One chapter provides protocols on the absolute quantification of protein safety biomarkers by mass spectrometry. Given that there is a growing interest to explore microRNAs as potential novel safety biomarkers in biofluids, several new chapters have been included to describe how next-generation sequencing, quantitative RT-PCR, and in situ hybridization technologies can be applied to investigate microRNAs for this purpose. A new chapter has also been added on the isolation and characterization of urine exosomes, which can potentially be used to detect nephrotoxicity.

I would like to thank all the contributing authors for providing state-of-the-art procedures, detailed protocols, and tips and tricks to avoid pitfalls. I am grateful to the series editor, John Walker, for inviting me to edit this second edition of the volume. The result is a compendium of analytical technologies, including some review chapters, with a focus on clarity and applicability in real life laboratory practice. The intended audience mainly consists of pharmaceutical scientists, toxicologists, biochemists, and molecular biologists, and anyone else with a specific interest for a method used in drug safety evaluation that could be used in other disciplines.

Vitry-sur-Seine, France *Jean-Charles Gautier*

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