Springer Protocols

Methods in Molecular Biology 691

Drug Safety Evaluation

Methods and Protocols

Edited by
Jean-Charles Gautier

Humana Press

Drug Safety Evaluation

Methods and Protocols

Edited by

Jean-Charles Gautier

Disposition, Safety and Animal Research, sanofi-aventis, Vitry-sur-Seine, France

Humana Press

Editor
Jean-Charles Gautier
Disposition, Safety and Animal Research
sanofi-aventis
Vitry-sur-Seine
France
jean-charles.gautier@sanofi-aventis.com

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-60327-186-8 e-ISBN 978-1-60761-849-2
DOI 10.1007/978-1-60761-849-2
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010937423

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

Non-clinical 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 to 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.

General toxicity studies are usually performed in a rodent and in a nonrodent species to determine target organs of toxicity and evaluate doses of a new drug candidate that can be safely administered to man. In this book, specific aspects related to the experimental design of toxicity studies conducted to support drug combinations in humans and pediatric indications are described in the reviews of Chaps. 1 and 2, respectively. In general toxicity studies, the key traditional endpoints evaluated include clinical signs, clinical pathology parameters, along with macroscopic examination of organs at necropsy and light microscopic examination of a comprehensive list of tissues. Chapter 3 details the necropsy and sampling procedures used in rodents, and Chap. 4 highlights the histopathology procedures from tissue sampling to histopathological evaluation. Chapters 5 and 6 describe additional methods, such as immunohistochemistry, tissue microarrays, and digital image analysis, which can be used to complete and refine the traditional histopathological examination of organs.

Genotoxicity studies are carried out to evaluate the potential of new drug candidates to induce mutations and/or chromosomal damages. Chapter 7 presents the method of the micronucleus assay and its combination with centromeric labeling in the fluorescence *in situ* hybridization (FISH) technique to detect aneugenic events. Chapter 8 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.

Safety pharmacology studies are conducted to evaluate the effect of compounds on the cardiovascular, respiratory, and central nervous system functions before the first administration to humans. Chapter 9 describes a manual patch-clamp technique used to study the effect of compounds on the HERG cardiac K^+ channel in order to evaluate the potential to induce "torsades de pointe", an arrhythmic disorder that can be fatal in humans.

When unexpected toxicity arises during these studies, it is important to investigate the mechanisms of toxicity and assess the potential translation to humans. Traditional histopathological examination of target organs and clinical pathology parameters are sometimes in default, and novel 'omics technologies, such as transcriptomics, proteomics, and metabonomics could allow to generate new hypotheses on the mechanisms of toxicity. Detailed protocols related to these 'omics technologies are presented in Chaps. 10–12.

Of note, the gene expression results obtained via transcriptomics experiments need to be confirmed by quantitative RT-PCR. However, accurate interpretation cannot be performed without proper statistical analysis of RT-PCR data. Chapter 13 examines some of the issues concerning RT-PCR experiments that would benefit from rigorous statistical treatment.

In vitro functional assays can be used to elucidate mechanisms of toxicity in the context of drug safety evaluation. Chapter 14 describes an *in vitro* assay used to evaluate the effect of compounds on the mitochondrial respiration chain in cultured rat hepatocytes. Mitochondrial dysfunction is indeed a major mechanism, whereby drugs can induce liver injury and other serious side effects, such as lactic acidosis and rhabdomyolysis, in some patients. *In vitro* assays can also be used during the early phase of drug development to screen compounds for their potential to induce developmental toxicity. This is illustrated with the Fetax and the zebrafish models in Chaps. 15 and 16, respectively. Drug-induced toxicity is often associated with the formation of reactive metabolites that bind covalently to proteins. Chapter 17 describes *in vitro* assays used at the lead optimization stage of drug discovery to evaluate the potential of drug candidates to bind covalently to proteins by incubating a radiolabeled analog of the compound with liver microsomal preparations or whole cells. Sophisticated mass spectrometry-based methods can also be used to identify chemical-adducted proteins both *in vitro* and *in vivo*. This is illustrated with specific examples in Chaps. 18–21.

Another developing field in drug safety evaluation is the identification and qualification of novel safety biomarkers that can be used to better monitor potential toxicity in both preclinical and clinical studies. Ideally, these new safety biomarkers should be more sensitive and/or specific than the traditional clinical pathology parameters and should be measurable in accessible fluids, such as plasma and urine. Chapters 22–24 provide sophisticated methods to discover new safety biomarkers using proteomics and metabonomics approaches. A protocol to quantify potential protein safety biomarkers by mass spectrometry is also described in Chap. 25.

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 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 in methods used in drug safety evaluation that could be translated to other disciplines.

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

Contributors

Alexander Amberg • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Hattersheim, Germany*

Stéphane Barbellion • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*

Paul C. Barrow • *Ricerca Biosciences, Les Oncins, Saint-Germain sur l'Arbresle, France*

Gabriel Baverel • *Metabolys Inc, Lyon Cedex, France*

Xiao-Liang Chen • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Bridgewater NJ, USA*

Andrew R. Collins • *Department of Nutrition, Faculty of Medicine, University of Oslo, Oslo, Norway*

Ben C. Collins • *UCD School of Biomolecular and Biomedical Science and Proteome Research Centre, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*

Emmanuelle Com • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France; High-Throughput Proteomics Core Facility, Biogenouest, Rennes, France*

Martine Courcol • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*

Stephen H. Day • *Department of Drug Metabolism and Pharmacokinetics, Merck Frosst Centre for Therapeutic Research, Kirkland QC, Canada*

Ilse Decordier • *Laboratorium voor Cellulaire Genetica, Vrije Universiteit Brussel, Brussels, Belgium*

Frank Dieterle • *Molecular Diagnostics, Novartis Pharma AG, Basel, Switzerland*

Agnès Duplany • *Metabolys Inc., and Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*

Maha El Hage • *Metabolys Inc., and Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*

Hassan Faiz • *Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*

Bernard Ferrier • *Metabolys Inc., and Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*

Laurence Fiette • *Human Histopathology and Animal Models Unit, Institut Pasteur, Paris, France*

Ashley A. Fisher • *Department of Pharmacology and Toxicology, Southwest Environmental Health Sciences Center, Center for Toxicology, College of Pharmacy, University of Arizona, Tucson AZ, USA*

William M. Gallagher • *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*

Catherine Gauthier • *Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*
Jean-Charles Gautier • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*
Olivier Gillardeaux • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Porcheville, France*
Philip D. Glaves • *Molecular Toxicology Group, Safety Assessment Department, AstraZeneca Pharmaceuticals Ltd, Macclesfield, Cheshire, UK*
Albrecht Gruhler • *Novo Nordisk, Måløv, Denmark*
Stacey L. Harper • *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis OR, USA*
Allen N. Jones • *Department of Drug Metabolism and Pharmacokinetics, Merck Research Laboratories, RahwayNJ, USA*
Jiesheng Kang • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Bridgewater NJ, USA*
Richard Khan-Malek • *Preclinical Development Biostatistics, sanofi-aventis R&D, Inc, Bridgewater NJ, USA*
Micheline Kirsch-Volders • *Laboratorium voor Cellulaire Genetica, Vrije Universiteit Brussel, Brussels, Belgium*
Gilles Labbe • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*
Matthew T. Labenski • *Department of Pharmacology and Toxicology, Southwest Environmental Health Sciences Center, Center for Toxicology, College of Pharmacy, University of Arizona, Tucson AZ, USA*
Serrine S. Lau • *Department of Pharmacology and Toxicology, Southwest Environmental Health Sciences Center, Center for Toxicology, College of Pharmacy, University of Arizona, Tucson AZ, USA*
Thomas Y. K. Lau • *UCD School of Biomolecular and Biomedical Science and Proteome Research Centre, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*
Jean-Francois Léonard • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*
Jean-François Lévesque • *Department of Drug Metabolism and Pharmacokinetics, Merck Frosst Centre for Therapeutic Research, Kirkland QC, Canada*
Alberto Lodola • *ToxAdvantage, Noizay, France*
Laure Malesic • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Porcheville, France*
Jean-Pierre Marchandeau • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*
Guy Martin • *Metabolys Inc., and Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*
Raluca Mateuca • *Laboratorium voor Cellulaire Genetica, Vrije Universiteit Brussel, Brussels, Belgium*
Terrence J. Monks • *Department of Pharmacology and Toxicology, Southwest Environmental Health Sciences Center, Center for Toxicology, College of Pharmacy, University of Arizona, Tucson AZ, USA*

Isabelle Mouche • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Porcheville, France*
Laoighse Mulrane • *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*
Stephen R. Pennington • *UCD School of Medicine and Medical Science and Proteome Research Centre, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*
José A. Ramos-Vara • *Department of Comparative Pathobiology, Purdue University, West Lafayette IN, USA*
David Rampe • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Bridgewater NJ, USA*
Sophie Renault • *Metabolomics and Metabolic Diseases, Laennec Faculty of Medicine, INSERM Unit N° 820, Lyon, France*
Elton Rexhepaj • *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*
Björn Riefke • *Laboratory Diagnostics, Non-Clinical Drug Safety Evaluation, Bayer Schering Pharma AG, Berlin, Germany*
Alfred Ross • *Hoffmann LaRoche, Basel, Switzerland*
Denise Ryan • *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland*
Götz Schlotterbeck • *Hoffmann LaRoche, Basel, Switzerland*
Hans Senn • *Hoffmann LaRoche, Basel, Switzerland*
Mohamed Slaoui • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Vitry-sur-Seine, France*
Jeanne Stadler • *Toxicology Consulting, Tours, France*
Peter Stone • *Agilent Technologies, Cheadle, UK*
Ning Tang • *Agilent Technologies, Santa Clara CA, USA*
Robert L. Tanguay • *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis OR, USA*
Lisa Truong • *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis OR, USA*
Jonathan D. Tugwood • *Molecular Toxicology Group, Safety Assessment Department, AstraZeneca Pharmaceuticals Ltd, Macclesfield, Cheshire, UK*
Ying Wang • *Disposition, Safety and Animal Research, sanofi-aventis R&D, Inc, Bridgewater NJ, USA*

Contents

Part I

General Toxicology

Chapter 1

Developing Combination Drugs in Preclinical Studies

Alberto Lodola

Abstract

Although combination drugs have been available for many years, it is only recently that preclinical guidelines have been released by the Food and Drugs Administration (FDA) and EMEA and as yet they are not part of the ICH process. In addition, the World Health Organisation and FDA have issued guidelines for combination drugs developed specifically to treat HIV infections. Depending on the type of combination (marketed drug/marketed drug; marketed drug/NME and NME/NME), the scope and complexity of studies can vary greatly. In all cases, however, a key issue is the potential for pharmacokinetic and/or toxicologic interaction between the components. For a marketed drug/marketed drug combination, a detailed review of the preclinical data available may suffice; particularly when the components have a history of co-administration at about the same dose and ratio as that of the proposed combination. For a marketed drug/NME combination, in addition to a review of the data for the marketed drug, a full ICH programme of studies will be required for the NME, and a study of up to 90 days duration (in one species) for the combination. With an NME/NME combination, each component will require a full ICH battery of studies and a combination study in one species. In all cases, additional studies may be needed to address data gaps. Given the many novel and complex issues that arise when developing combination drugs, we recommend that, whenever possible, the preclinical study strategy is discussed with the regulatory authorities.

Key words: Combination drugs, Regulatory guidelines, Preclinical issues, Study design, Dose selection, Development strategies

1. Introduction

The US Food and Drugs Administration (FDA) defines "Combination Drugs" (1) as follows:

- Co-packaged products (two or more separate drugs packaged together);
- Adjunctive therapy (when a second drug is used together with the drug for primary treatment);

Jean-Charles Gautier (ed.), *Drug Safety Evaluation: Methods and Protocols*, Methods in Molecular Biology, vol. 691,
DOI 10.1007/978-1-60761-849-2_1,

- Fixed dose combinations (two or more drugs combined in a single pill).

These drugs should not be confused with "Combination Products" which are defined in 21 CFR3.2(e) (2). A brief description of the development of "combination products" has been provided by Segal (3) and Portnoy and Koepke (4).

In this chapter, we discuss the preclinical development of "combination drugs." These drugs have been available in our pharmacies for many years; for example in 1975 in West Germany greater than two-thirds of the drugs on the official list were fixed combinations (5). In 1982 it was reported that about half of all marketed drugs in the USA were fixed combinations (6). Combination drugs are used in the treatment of a range of illnesses (7–10). Their major disadvantage is that neither the dose nor the ratio of the individual components can be varied by the physician. However, for patients on multiple medication, a combination drug simplifies treatment and hence compliance, given that fewer pills are taken there may also be a cost advantage (11). Combination therapy may also be more effective than monotherapy or have an improved safety profile. In Stage-I or -II hypertension, monotherapy may only produce a modest effect on blood pressure while a combination drug can produce a more significant reduction, given that each component blocks different effector pathways. Similarly, the incidence of peripheral oedema, linked to the use of calcium channel antagonist therapy, is reduced when co-administered with an ACE inhibitor (7).

2. Regulatory Guidance

The "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use" (ICH) (12) has defined the preclinical data requirements for monotherapy development. These requirements are an essential backcloth when assessing the preclinical needs for combination drug development.

2.1. Guidance for Monotherapy Development

Current guidance for monotherapy development is described in a range of guidelines (12) which are detailed in Table 1. In addition to data from these studies, "special studies," developed in the light of the emerging preclinical and clinical data, may also be needed to address specific issues. The relevance of these documents to combination drug development is twofold. First, for marketed drugs they are the reference against which the preclinical data for the marketed drug, often produced pre-ICH, are judged. Second, they define the studies and data needed for the

Table 1
List of current preclinical guidance documents available for the development of monotherapies (12)

ICH guideline	Topic
S1A	Need for carcinogenicity studies of pharmaceuticals
S1B	Testing for carcinogenicity of pharmaceuticals
S1C(R1)	Dose selection for carcinogenicity studies of pharmaceuticals and limit dose
S2A	Guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals
S2B	Genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals
S3A	Note for guidance on toxicokinetics: the assessment of systemic exposure in toxicity studies
S3B	Pharmacokinetics: guidance for repeated dose tissue distribution studies
ICH	Single dose toxicity tests
S4	Duration of chronic toxicity testing in animals (rodent and non-rodent toxicity testing)
S5(R2)	Detection of toxicity to reproduction for medicinal products and toxicity to male fertility
S6	Preclinical safety evaluation of biotechnology-derived pharmaceuticals
S7A	Safety Pharmacology studies for human pharmaceuticals
S7B	The non-clinical evaluation of the potential for delayed ventricular repolarisation (QT Interval prolongation) by human pharmaceuticals
S8	Immunotoxicity studies for human pharmaceuticals
M3(R2)	Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

novel component of a combination drug and guide the choice of studies with the combination.

2.2. Guidance for Combination Drug Development

Currently guidelines for combination drugs are nationally based and not part of the ICH process. The FDA provides specific guidance based on the preclinical requirements for three combination drug scenarios; combinations of marketed drugs, combinations of marketed drugs and new molecular entities (NME) and combinations of NMEs. Data requirements are summarised in Table 2.

In general, for marketed drugs, and if the components have a history of concomitant use at about the projected ratio little, or no, preclinical work is needed. For combinations involving NMEs, a full ICH package of studies is needed for the NME (see Subheading 2.1) and additional studies with the combination.

Table 2
Summary of the key points from the FDA Guidance for Industry (2006) Nonclinical Safety Evaluation of Drug or Biologic Combinations (1) for combinations involving NMEs

	Combination type		
Study	**MD/MB + NME**	**NME + NME**	**Comment**
Genetic toxicology	On the NME Combination	On the NME Combination	Not required for MD/MB if data consistent with modern requirements Generally not required
PK/ADME and toxicokinetics	On the NME	On the NME Combination	Per ICH-M3 and/or ICH-S6 If same target organ/system
Safety pharmacology	On the NME Combination	On the NME Combination	Per ICH-M3 and/or ICH-S6 If same target organ/system
General toxicology	On the NME Combination	On the NME Combination	Per ICH-M3 and/or ICH-S6 Study of up to 90-days duration
Reproductive and development toxicology			
Fertility (Study 1)	On the NME	On the NME	Per ICH-M3
Implantation/early development (Study 2)	On the NME	On the NME	Per ICH-M3
Embryo-foetal development (Study 3)	On the NME Combination	On the NME Combination	Per ICH-M3 Unless the MD or NME is pregnancy category "D" or "X"
Carcinogenicity	On the NME	On the NME	Generally not needed for the combination if NMEs tested
Animal models of efficacy	Generally not needed	Generally not needed	
Further studies	As required to address data gaps/specific issues	As required to address data gaps/specific issues	

Abbreviations: *MD* marketed drugs, *MB* marketed biologics, *NME* new molecular entity