Chapter 1
Introduction: An Overview of Industry and Regulatory Perspectives on the Genotoxic and Carcinogenic Assessment of Pharmaceuticals

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Abstract While there are numerous manuscripts and review articles that cover various aspects of genotoxicity and carcinogenicity testing of pharmaceuticals, there is no single text book that brings all of these concepts together in a practical way. Therefore, the intent of this book is to help industry scientists and regulators develop a more comprehensive understanding of the concepts and strategies used to assess risk of these critical components of a nonclinical testing program. Assessing the risks for genotoxicity and carcinogenicity of pharmaceuticals differs from other chemicals since pharmaceuticals are given intentionally at relatively high doses in order to achieve a therapeutic benefit. Therefore, the safety assessment of pharmaceuticals, including genotoxicity and carcinogenicity evaluations, is often based on defining acceptable therapeutic margins and establishing the human relevance of findings in the animal studies. This book focuses on these topics in an integrated way, taking into account the rapid advances in safety sciences and evolving regulatory requirements. The book is written by well recognized experts from the pharmaceutical industry and US and European health authorities. All of the authors have either addressed various nonclinical safety issues over the course of their careers, were involved in developing the testing guidelines, and/or are thought leaders that continue to drive the science of toxicology forward. The order of the chapters reflects the usual sequence of genotoxicity and carcinogenicity testing in the pharmaceutical industry, starting with structure-based assessments very early in the drug development process. The book is also intended help readers better understand and appreciate the complexity of the regulations and breadth of toxicology research that are necessary to support the development of new drugs. Developing new drugs is extremely difficult as the expectations for safety continue to increase and target biology becomes more complex. These factors combined with the pressure to reduce animal use makes nonclinical safety testing challenging in today's environment. The last few years indicate that we are

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at the cusp of major changes in the nonclinical safety testing of pharmaceuticals as evidenced by the number of new and revised ICH guidances along with advances in the development and application of in vitro safety assays. This book attempts to bring it all together as a "state of the science" and practical guide with references to numerous examples and important case studies. The Introduction provides a brief overview of each chapter and highlights some of the key considerations and approaches for de-risking drug development programs.

Keywords Carcinogenicity • Genotoxicity • ICH • Pharmaceuticals • Rodent bioassays

The realization that exposure to certain chemicals could lead to cancer originated well over a century ago based on observations of increased testicular cancer in chimney sweeps and increased urinary bladder cancer in workers in the dye industry. This was followed decades later by clear experimental evidence of chemical-induced tumors in animal studies following topical or oral administration of coal tar, polycyclic aromatic hydrocarbons, 2-naphthylamine, and azo dyes. A detailed history of chemical induced carcinogenesis can be found in numerous review articles and textbooks [1–5].

In response to the growing appreciation that some chemicals could lead to cancer and the need to protect public health, the US government enacted legislation in 1962 that required drug manufacturers to prove that their products were both safe and effective prior to marketing approval [6]. Around that time, the FDA also produced the first set of guidelines for preclinical safety testing [7]. Although these initial guidelines did not specifically state the need for carcinogenicity testing, chronic studies up to 18 months were recommended. Over the next few decades, protocols for carcinogenicity studies were refined and standardized. However, even today, they are largely based on the protocols developed by the NCI in the 1960s [7, 8]. Ironically, despite all of the technical and scientific innovations over the last 50 years, a 2-year study in rats is still considered the gold standard for carcinogenicity testing by regulatory authorities [9].

By the 1970s it was generally accepted that the mechanism of chemical-induced carcinogenesis involved interaction of the chemical with host DNA either by direct binding of the parent molecule or through the formation of reactive intermediates by the cytochrome P450 drug metabolizing enzyme system [1, 3, 10–12]. If not repaired, DNA binding of these reactive chemicals could lead to mutations in the genetic code and, ultimately, transformation of normal cells to cancer. Although this is an overly simplified description of chemical-induced carcinogenesis, it is evident that the early events in this process could be investigated without the use of animals. Accordingly, Bruce Ames et al. developed a relatively simple in vitro assay to detect chemical mutagens using S*almonella typhimurium* bacteria and a mammalian drug metabolizing enzyme system [13]. It is now generally accepted that the "Ames assay," as it is commonly known, can detect DNA-reactive carcinogens with a fairly high degree of concordance [14–20]. Based on this high degree of sensitivity for identifying multi-site and multi-species carcinogens, the Ames assay is used within

the pharmaceutical industry as an early screening assay and is part of the core battery of genotoxicity tests required by regulatory authorities [21, 22]. With some exceptions (e.g., cytotoxic anticancer drugs), almost all positives in this assay are dropped from development.

While the current pharmaceutical testing paradigm for genotoxicity and carcinogenicity testing generally works well, it has not changed dramatically for several decades and there is a growing interest in developing new assays and predictive tools. Science and technology are constantly evolving and the possibility of predicting the carcinogenicity of chemicals based on structure and/or molecular signatures is gaining attention. Although more accurate predictions of human safety will ultimately drive the application of these new tools, the pressure to reduce resources and minimize animal testing are also very real and directly contribute to the growing interest and application of alternative approaches. In Chap. 2, Lidya Stavitskaya, Jiri Aubrecht, and Naomi Kruhlak describe the current state of efforts by industry and FDA to use structure- and biology-based models to predict the mutagenicity and carcinogenicity of pharmaceuticals. The application of computational methods to evaluate the relationship between chemical structure and genotoxicity/carcinogenicity is relatively inexpensive and does not require actual chemical synthesis for testing. Therefore, quantitative structure-activity relationship (QSAR) models are being used more routinely in the early drug development process and for predicting mutagenicity of drug substance impurities. In contrast to QSAR, biology-based predictive models such as toxicogenomics are often used as investigative tools to address questions on the human relevance of findings. Whether these new biology-based models get incorporated into the standard mutagenicity/carcinogenicity testing paradigm either as replacements for any of the current studies or, more likely, as supplemental/supportive information will depend on further refinements, robust validation, and larger databases.

In Chap. 3, Laura Custer and Mark Powley describe the application and interpretation of the Ames assay as well as other in vitro and in vivo tests that are used to assess the potential genotoxicity of pharmaceuticals as described in International Conference on Harmonization (ICH) S2 (R1) [21]. [ICH is an organization involving regulators and research based industries from US, Europe, and Japan which was founded in 1990 to improve the efficiency of pharmaceutical R&D by developing and implementing harmonized guidelines and standards]. Some genotoxicity test results can be fairly straightforward. For example, a clear positive in the Ames assay would likely lead to a quick decision to terminate development of that compound. On the other hand, a small increase in micronuclei formation or chromosome aberrations relative to controls might require some follow up studies to put that finding into better perspective for human safety assessment. Laura Custer and Mark Powley review the different tests and strategies to de-risk these situations using a weight-of-evidence approach from both a regulatory and industry perspective.

In addition to the standard battery of genotoxicity tests that are required by regulatory authorities as outlined in ICH S2 (R1), there are number of new in vivo genotoxicity assays that are being developed to supplement and/or potentially substitute for the core battery. These new tests include the comet assay, the Pig-A gene mutation

assay, and the liver micronucleus test [23–28]. In Chap. 4, Patricia Escobar, Stephen Dertinger, and Robert Heflich provide an overview of each of these tests, including their value and limitations as investigative tools in regulatory testing. The authors also briefly discuss strategies for de-risking positive findings in the core battery of genotoxicity assays and the re-emergence and interest in the transgenic rodent gene mutation assay for evaluating germ cell mutagenicity.

In addition to fully characterizing the genotoxicity and carcinogenicity of the active pharmaceutical ingredient, it is also necessary to assess the potential genotoxicity of process impurities and degradants. For context, all pharmaceutical impurities have to be identified, qualified, and controlled at certain threshold levels [29–31]. However, a lingering concern is that there may be genotoxic impurities below these threshold levels that could still lead to an increased and unacceptable risk for carcinogenicity. How to deal with these low level genotoxic impurities has been a challenging and frustrating issue for industry and regulatory scientists for a number of years largely due to different views on the overall safety risks and by the complexity of the technical and synthetic process changes that are often required to control them [32–34]. For example, it is well accepted that humans are exposed to naturally occurring carcinogens almost every single day of their lives through diet, lifestyle, and sunlight. So, what level of increased carcinogenic risk is considered negligible and how does that level of risk translate to a safe level of a mutagenic impurity? In addition, since the electrophilic nature (and inherent biological reactivity) of chemicals is highly variable and dependent on their unique structure, the potential carcinogenic risk cannot be the same for all mutagenic chemicals. So, recognizing that a global guidance was needed to standardize the criteria and control strategies for genotoxic impurities, industry and health authorities agreed to establish an ICH Expert Working Group (EWG) in 2010 to develop an international harmonized guideline. In Chap. 5, Peter Kasper and Lutz Muller, who were both members of this EWG discuss the history and concepts of the new and important ICH guidance on DNA reactive (mutagenic) impurities that was published in 2014 [35]. The authors also provide a few examples on how the principles of this guidance have been interpreted and applied in real world situations.

If the pharmaceutical industry can effectively screen out DNA reactive compounds and de-risk other potential genotoxic drugs with more sophisticated and relevant models, why is there a need to conduct 2-year rodent carcinogenicity studies and why are there so many positive findings in these studies, especially in the labels of approved drugs? Are tumor findings in rodents relevant to humans? While a deep dive into the mechanisms of non-genotoxic carcinogens is outside the scope of this book, it is clear that most drugs associated with tumors in animal studies are not DNA-reactive. For example, various hormones and growth factors can cause tumors in animals due to prolonged and exaggerated pharmacological effects at high doses [36–38]. Immunosuppressive drugs can lead to an increase in viral associated tumors in both animals and humans [39–41]. In fact, any drug that causes tissue hyperplasia in animals could be considered a suspect carcinogen (until proven otherwise) since increased cellular proliferation has long been recognized as a characteristic of tumor promotion and progression [42–44]. Therefore,

it is not a question of whether there are non-genotoxic rodent carcinogens but rather are any of these considered relevant to humans. We know from decades of research in toxicology that many tumor findings in animals are not relevant to humans due to unique characteristics of rodent physiology and their subsequent response to chemicals. So, if we had readily accessible, sensitive, and specific biomarkers of carcinogenicity, humans could be monitored for these changes in clinical trials. Unfortunately, such biomarkers do not currently exist and the collection of most tissue samples from humans to investigate evidence of tissue hyperplasia is, of course, unreasonable. So, while there has been and continues to be an abundance of scientific debate on the predictive value of rodent carcinogenicity studies, especially in the pharmaceutical industry [45–50], rodent carcinogenicity studies are still conducted for most drugs, especially small molecules.

In Chap. 6, James MacDonald and David Jacobson-Kram provide a brief historical overview of the regulations regarding pharmaceutical safety testing, what we've learned from decades of rodent bioassay studies, what alternative testing approaches have been considered, and how carcinogenicity assessments may be refined in the future. The authors introduce an ongoing ICH initiative that is designed to test the ability of sponsors and Drug Regulatory Agencies (DRAs) to prospectively predict the outcome of 2-year carcinogenicity studies based on toxicology, pharmacology, and mechanistic endpoints. It is expected that a successful outcome of this exercise (i.e., the ability predict the results of carcinogenicity studies with a high degree of certainty) may lead to changes in the carcinogenicity testing requirements for small molecules in certain cases. More details on this ICH initiative are covered in Chap. 7. Other important topics covered in Chap. 6 include: (1) a description of how carcinogenicity study protocols and study results are reviewed by the FDA; (2) the role of the Carcinogenicity Assessment Committee (CAC) and Executive CAC in this process; and (3) the regulatory expectations in regards to the design and analysis of these studies.

In Chap. 7, Frank Sistare and Abby Jacobs cover four main topics including: (1) the current global regulatory requirements for carcinogenicity testing of small molecules and the limitations of these approaches; (2) numerous examples where positive rodent carcinogenicity study outcomes were not considered relevant to humans; (3) the increasing use of the 6-month transgenic rasH2 mouse model as part of the standard carcinogenicity testing paradigm; and (4) the ongoing effort within ICH to potentially reduce the number of 2-year rat carcinogenicity studies for small molecules. The global carcinogenicity testing requirements for small molecules is covered in a series of documents developed through ICH including ICH S1 (the need for long-term rodent carcinogenicity studies of pharmaceuticals) [51], ICH S1B (testing for carcinogenicity of pharmaceuticals) [9], and ICH S1C (R2) (dose selection for carcinogenicity studies) [52]. As mentioned previously, the ongoing initiative within ICH to potentially change the carcinogenicity testing paradigm for small molecules involves a prospective analysis of ongoing carcinogenicity studies by both sponsors and DRAs to determine how well the outcome of these studies can be predicted. The rationale for this initiative was supported by the results from a retrospective analysis of carcinogenicity studies conducted by the pharmaceutical

industry which showed that almost 85 % of rat carcinogenicity study outcomes could be predicted by the mechanism of action of the drug and by the results from earlier nonclinical safety studies [53]. It was also estimated that almost 40 % of 2-year carcinogenicity studies could be avoided if no signals were detected after assessing these criteria. While this is a relatively high predictive value, especially considering the diverse range of drugs, it is not 100 % and a number of questions were raised by Health Authorities from US, Japan, and Europe. The main concern from regulators was about the 15 % of drugs that were not correctly predicted by this paradigm. So, the question to be answered was: if this process was followed, how many potential human carcinogens (false negatives) would "slip" through the system? Of course, the real answer is dependent upon whether one believes that any false negatives in the pharmaceutical industry data analysis represent true human carcinogens. In all these cases the drugs were approved anyway and, for most of the false negatives, there was a mechanistic explanation (e.g., species specific effect) or exposure margin that invoked no human relevance. Nevertheless, since regulators are charged with protecting human health, it is not hard to understand why any recommendation from the industry to eliminate the "gold standard" for carcinogenicity assessment would face some scrutiny.

However, despite the reluctance from health authorities to accept the industry proposal, the ability to predict the outcome of rat carcinogenicity studies is not without merit and the EMA, FDA, and PMDA ultimately agreed to participate in the prospective ICH study to test the industry hypothesis using a set of standardized criteria. It is expected this study will generate enough information in a real world situation so that health authorities can determine the ability of both sponsors and regulators to predict the outcome of the rat carcinogenicity study. Pending a successful outcome of this initiative, EMA, FDA, and PMDA agreed to consider revising ICH S1 and allow a waiver of 2-year rat carcinogenicity studies under certain circumstances.

In Chap. 8, Maggie Dempster et al. discuss the carcinogenicity testing of biopharmaceuticals which is included in ICH S6 (preclinical safety evaluation of biotechnology-derived pharmaceuticals) [54]. There are many distinct differences between small molecules and biopharmaceuticals with respect to their physicochemical and biological properties and these differences must be understood and appreciated in order to conduct the most appropriate carcinogenicity assessment. This is especially true for biopharmaceuticals that are not biologically active in rodents. In this chapter, Dempster et al. review the different classes of biopharmaceuticals such as growth factors and immunosuppressive drugs that have been associated with an increased tumorigenic risk in humans simply based on their pharmacology. The authors also present some case studies to show different approaches for evaluating the carcinogenic risk of biopharmaceuticals and in translating these findings to humans, including the use of pharmacovigilance data.

By necessity, 2-year carcinogenicity studies are conducted relatively late in drug development and neither sponsors nor regulators can afford to deal with inadequate studies or uninterpretable results just prior to registration. While the ICH S1 initiative may lead to a reduction in the overall number of 2-year carcinogenicity studies

for small molecules, it will not eliminate them completely. In fact, it is estimated that for about half of all drugs, there will be enough uncertainty with respect to the predicted carcinogenicity outcome that a 2-year rat study will be required. So, the big question is what happens when you actually get a carcinogenic signal in your study? Does it matter? The answer depends on a number of factors including the strength of the tumor signal, the exposure margin (relative to the AUC at the recommended human dose), and the known relevance to humans. Regardless, a statistically significant tumor finding in a carcinogenicity study is a major event and can lead to unacceptable delays in development and marketing approval, and possibly even termination of the project.

In Chap. 9, Todd Bourcier and Denis Roy discuss what factors need to be considered in identifying and de-risking the human relevance of tumor findings in 2-year rat carcinogenicity studies. From the industry perspective, a positive signal in a 2-year rat carcinogenicity study has a huge business and financial impact, especially considering that these studies are generally conducted late in development to support marketing submissions. Given that the average development time of a drug is about 10 years and can cost > $2 billion dollars of R&D investments [55, 56], this is not the time to uncover major approvability issues. Of course, a positive rodent carcinogenicity study also puts regulators in a difficult situation since they do not want to withhold approvals of new medicines for a finding that may not be relevant to humans but, at the same time, they cannot take risks with protecting human health. Unfortunately, despite the best attempts to de-risk the carcinogenicity potential of new drugs, surprises do happen. At that point, the burden is mostly on industry scientists to propose a rationale scientific argument for why the finding is not likely relevant to humans. This may include conducting follow-up mechanistic and investigative studies to put the carcinogenicity finding into proper perspective and provide additional experimental evidence to support their hypothesis. Bourcier and Roy review the factors that need to be considered when designing and interpreting carcinogenicity studies. They also offer additional suggestions and guidance on how to manage and communicate carcinogenicity findings to internal and external stakeholders including a case study on the GLP-1 receptor agonists.

However, if the carcinogenicity studies are negative (i.e., no statistically significant increase in tumors in the treated groups), then the presumption would be that the molecule has essentially been de-risked as a carcinogen. (Note: some exceptions would include hormonal agents and immunosuppressive drugs where an increase in tumors may not be evident in the carcinogenicity studies, but where the concern for carcinogenicity may still exist based on the mechanism of action). Unfortunately, like all things in life, nothing is 100 % guaranteed and "stuff" happens. This is especially true in clinical trials where imbalances in tumor incidences can occur between treatment groups due to random chance and more rigorous medical examination of the subjects. The imbalance in tumor incidence can occur in either direction for the treatment group when compared to the controls but safety concerns are only raised when the incidence of a particular tumor in the treated group is increased. This is true even if there is no statistically significant difference in overall tumor incidence between the groups. As one can surmise, this is a very challenging situation and can

lead to speculation of a possible tumor promotion effect. There are no well characterized or established tumor promotion models, and whether a "tumor promoter" can actually make it all the way through a toxicology program, including a clean carcinogenicity study without some signal is debatable, in and of itself. Nevertheless, this situation has happened more than once and in Chap. 10, Lorrene Buckley, Beatriz Silva-Lima, and Mark Tirmenstein discuss how a positive tumor signal in a clinical trial is determined and what kind of additional follow up investigative studies can be performed to further de-risk the concern. These follow up investigations must be designed on a case-by-case basis and with a very strong scientific rationale to fully interrogate biological plausibility, including an assessment of tumor promotion and progression. These latter assessments are especially critical given that tumors in clinical trials are not likely to arise de novo from drug treatment given the relatively short latency period. The authors briefly discuss a few models that can be used for studying tumor promotion and progression although it is well recognized that development of more relevant models is warranted. Finally, Buckley et al. present some important case studies in which clinical tumor findings were effectively de-risked by applying robust scientific arguments along with data from a few key follow-up investigative studies.

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