# Chapter 7
# Carcinogenicity Testing Strategies for Small Molecules

**Abigail Jacobs and Frank D. Sistare**

**Abstract** This chapter provides an overview of the current state of carcinogenicity testing strategies used to support marketing approvals of human small molecule pharmaceuticals. Testing strategies for biologic molecules is beyond the scope and the reader is referred to Chap. 8 by Dempster et al. In this chapter a brief history of pharmaceutical carcinogenicity testing is summarized that describes the path of evolution to our current state. The current state of pharmaceutical carcinogenicity testing strategy as defined by internationally agreed upon ICH guidelines is reviewed, including the use of transgenic mouse models in pharmaceutical carcinogenicity testing strategies. Limitations of these current testing approaches are summarized and examples are used to describe and explain the implications and impact of such limitations on practical aspects of pharmaceutical development. Often times, approaches are successfully deployed by industry scientists to support conclusions that positive rodent carcinogenicity study outcomes are related to compound class effects and are not human relevant, and examples are provided where product marketing has been enabled. Finally based on decades of such repeated experiences, a vision for a near future state pharmaceutical carcinogenicity testing strategy is described where the burdens of carcinogenicity testing may be reduced without compromising human safety, and the steps in progress to realize that vision are summarized.

**Keywords** Pharmaceutical • Carcinogenicity Testing • Tg.rasH2 Transgenic Mouse • ICH Guidance

A. Jacobs
US Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD, USA

F.D. Sistare (✉)
Safety Assessment and Laboratory Animal Resources, Merck and Co., Inc., West Point, PA, USA
e-mail: frank.sistare@merck.com



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## 7.1 The Evolution of Pharmaceutical Carcinogenicity Testing

The majority of animal toxicology studies conducted to support marketing approval of human pharmaceuticals are designed to support the safe conduct of progressively longer duration clinical trials where human safety and efficacy of new drug candidates can be evaluated. Adverse findings seen only at very high exposure margins in animal toxicology studies are generally of low concern for humans. Adverse findings that are monitorable and reversible and are of questionable human significance, can most times be more definitively evaluated in clinical studies. Two types of animal toxicology studies, however, are conducted not to support clinical investigation of human safety, but rather serve as surrogates for human safety. Those are the animal studies that are conducted to assess carcinogenic potential and the animal studies conducted to assess developmental and reproductive toxicology potential. Carcinogenicity studies and developmental toxicology studies in animals are intended to reveal the likely effect expected of drug administration under relevant conditions of human use. Nevertheless, these animal studies are conducted under conditions that are designed to both pressure test and provoke evidence for potential for such toxicities at high drug exposures, as well as at relevant exposures that may more closely match human use.

Therefore, because a true assessment of human carcinogenicity potential cannot be practically evaluated across all organs and tissues, in hundreds of humans after lifetime administration of a drug under relevant conditions of use, a pragmatic approach using animals was needed to serve as a surrogate of this carcinogenicity assessment for humans, to support marketing decisions. Examples exist of pharmaceutical companies conducting 7 or 10 year cancer studies in dogs or monkeys before the period of time between 1978 and 1982 when Good Laboratory Practices [19] were established, OECD Guidelines [42] were published, and the FDA Bureau of Foods published the Red Book [16]. However, testing in mice and rats has and continues to serve as the mainstay for pharmaceutical carcinogenicity evaluation. The conditions of human use requiring rodent carcinogenicity testing; the dose and exposure of drug (and metabolites) needed to fairly evaluate a drug's human carcinogenicity potential; the duration of testing; and the species needing to be tested have each undergone evolution over the past 30+ years. Current carcinogenicity testing guidelines defining agreements reached in each of these areas were established following the launch of the International Conference on Harmonization in 1990. Negotiations for defining current regulatory carcinogenicity testing expectations for pharmaceuticals with revisions implemented through international negotiation, as supported by collective experience and data, have been described recently [50]. The three current ICH guidelines, namely S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals [26], S1B Testing for Carcinogenicity of Pharmaceuticals [27], and S1C(R2) Dose Selection for Carcinogenicity Studies of Pharmaceuticals [28]

provide recommendations on which pharmaceuticals warrant carcinogenicity testing, appropriate approaches for evaluating carcinogenicity potential, and appropriate dose selection, respectively.

The current ICHS1A guideline discusses the criteria used to determine whether an evaluation of the carcinogenic potential of a pharmaceutical is considered necessary. The guideline treats pharmaceuticals differently based on duration of exposure, establishing that for small molecule human pharmaceuticals, animal carcinogenicity studies are needed for drugs that would be used continuously, or repeatedly and intermittently for greater than 6 months. Furthermore, even for pharmaceuticals used for short durations, carcinogenicity studies may be needed when a priori concern about carcinogenic potential exists, which could include for example, chemical structure, previous compound class experience, evidence of preneoplasia in shorter term animal studies, or long-term tissue retention. ICHS1A discusses clinical duration and exposure, causes for concern, genotoxicity, route of exposure and extent of systemic exposure, and endogenous peptide and proteins and analogs. In addition ICHM3R2 [29] clarifies when in drug development that the studies should be conducted, i.e., generally to support marketing, and rarely to support clinical trials. It notes that for pharmaceuticals developed to treat certain serious diseases for adults or pediatric patients, carcinogenicity testing, if recommended, can be concluded post-approval. Some parts of ICHS1A, such as discussions of photocarcinogenicity, have been superseded by ICHM3R2 and ICHS10 [32], which no longer recommend such studies.

The current S1B guideline discusses the experimental approaches intended to assess carcinogenic potential of a pharmaceutical when such an evaluation is indicated by the criteria discussed in S1A. The S1B guideline effectively treats pharmaceuticals equally in recommending that all drugs needing carcinogenic assessment be evaluated in a 2-year rat bioassay and a 2-year or shorter term mouse bioassay. ICH S1B establishes that two species should be studied, at least one of which should be a 2-year study. It also mentions other in vivo models, such as models of initiation-promotion in rodents or models of carcinogenesis using transgenic or neonatal rodents. In the past 18 years, initiation-promotion models have not been accepted as replacements for the second species, but the use of certain transgenic and neonatal mouse models has become accepted in the United States. This guidance opened the door for a 6-month transgenic mouse study to fill the need of the second species study, used in conjunction with a 2-year rodent study, usually the rat. ICHS1B also provides general guidance on interpretation of the carcinogenicity studies pointing out the value of additional mechanistic studies to help address relevance of the results of carcinogenicity study findings to humans. Mechanistic studies have been very useful in assessing human risk from carcinogenicity findings in rodents. Cross-species receptor incidence and density for receptor-mediated effects and off-target effects, gene expression or microRNA expression, cross-species pathway analysis studies, and other studies used currently for assessment of human relevance are not specifically mentioned in the 18-year-old ICHS1B guidance.

ICH S1CR2 considers dose setting criteria for the high dose in 2-year carcinogenicity studies to include either: (1) an MTD based on toxicity endpoints, (2) a limit dose of 1500 mg/kg/day (for compounds not exceeding a daily human dose of 500 mg/day and when exposure margins of tenfold can be achieved), (3) pharmacokinetic endpoints specifying the need to reach a 25-fold exposure multiple over clinical exposure with criteria specified for comparisons of AUC in animals and humans, (4) a dose resulting in saturation of absorption, (5) pharmacodynamic endpoints that may limit high dose selection, or (6) a maximal feasible dose. All these criteria apply to studies in transgenic mice, except for pharmacokinetic endpoints. In this regard, for transgenic mouse studies there exists a data gap relating to mutual understanding and acceptance as to what would constitute a reasonable upper exposure limit to be considered an adequate test. As a result sponsors are sometimes facing a choice of conducting a transgenic mouse study at exposures that may reach hundreds-fold human exposure margins, or conducting a conventional 2-year mouse study at a 25-fold exposure margin, since regulatory position on this is evolving and presently unclear.

Although it has always been possible for a drug developer to request a waiver from carcinogenicity studies in the United States, (may be granted e.g., for short-term use, for life-threatening indications such as advanced cancer per ICHS9 [31], when values close to human exposures cannot be achieved in rodents, or for orphan drugs), ICHS6 addendum [30] specifically discusses when a biologic product can be labeled without the conduct of carcinogenicity studies. A drug developer can develop a case, based on various data sources, as to why a carcinogenicity study may not be warranted (e.g., a risk is already identified or lack of a risk seems clear, or the rodents don't have the pharmacologic activity). It is important to note that ICH Guidance S6 set the precedent allowing that for biological pharmaceuticals the opportunity exists for sponsors to explain why carcinogenicity testing would be inappropriate and waivers have been given for conducting such testing. In 2014, 10 of 11 requests for carcinogenicity study waivers of biologics were accepted by the FDA. Among the ten waivers granted are examples in each of three categories for not conducting the rodent carcinogenicity study – risk already identified, lack of risk, or rodent model is not scientifically relevant.

Generally, as more comfort has developed over time with the interpretation and understanding of recurring patterns of test outcomes, the burden of animal carcinogenicity testing for all pharmaceuticals may be expected to continue to decline. Prior to 2008, pharmaceuticals with evidence of genotoxicity could not invoke limit doses using the 25-fold exposure guidance for top dose selection. Since the most recent revision to these ICH Guidelines [28], which removed the 25-fold exposure top dose selection as a restriction for drugs with a positive genotoxicity test result, a number of publications have emerged proposing further ICH Guidance revision supporting a future state for small molecule pharmaceuticals that is analogous to that currently in place for biological pharmaceuticals under ICHS6. Such a future state is expected to reduce the resource burden needed to conduct, analyze, and report carcinogenicity studies, as well as address some limitations and imperfections of carcinogenicity testing without compromising protection of human safety.

## 7.2 Numerous Limitations and Imperfections of Rodent Carcinogenicity Testing Have Become Apparent Over the Years

Generally speaking the current approach as described above relying on rodents to assess pharmaceutical carcinogenicity risk potential to humans has met society's needs. One can argue that with very few exceptions rodents respond in an appropriately sensitive manner to all known human carcinogens. Immunosuppressants are variably tumorigenic in rodents, likely dependent on a variable presence of endogenous tumor virus in test animals. While arsenic and few other human carcinogens have been cited as not being convincingly carcinogenic in animals, questions of the adequacy of animal testing in such instances have been raised, and the reasonable statement has been made that "…no human carcinogens…have been tested in animals that have been shown to be unequivocally negative [25]." However, many drug-related rodent neoplasms may not be relevant to humans, especially for non-genotoxic drugs. For this reason, the specificity of the current rodent based pharmaceutical carcinogenicity testing approach has been called into question. Numerous examples of human irrelevance based on investigative toxicology study data have been made in many publications over the past 20 years, and in submissions to regulatory authorities as well, that have been used to support marketing decisions. Some explanations that have been accepted for drug-related rat carcinogenicity findings deemed of questionable human relevance to support regulatory decisions are described below.

## 7.3 Drug-Induced Rodent Tumors Can Be Associated with Intended Pharmacology

Some rodent carcinogenicity findings may be categorized as relating to the on-target intended pharmacologically mediated drug action, but human relevance is questioned because of the excessive and sustained nature of the pharmacologic manipulation and downstream consequences realized during the conduct of the study that are shown to be unique to the rat. Receptor distribution and potency (binding constant) can differ markedly across species, for example. Humans may have a low incidence of a receptor that is more prevalent in rats or mice, and thus would be less susceptible to effects seen in rodents under conditions of clinical use, e.g., GLP-1 agonists and thyroid C-cell neoplasms in rodents versus humans [5]. Uterine leiomyomas in mice can be caused by dopamine receptor agonists and the resulting decrease in levels of prolactin [3]. Leiomyomas of the mesovarium in rats caused by beta-2-adrenergic agonists are not thought to be relevant to humans under conditions of use [34]. Pancreatic acinar neoplasms in rats are considered to be secondary to chronic cholecystokinin stimulation, and rats are considered to be much more sensitive to this effect than are humans [21].

Mammary neoplasms may occur in rats secondary to decreases in dopamine signaling and increases in prolactin levels [20]. Sprague Dawley rats are considered to be more sensitive to this effect than humans. However, prolactin may also be increased for some of these drugs in humans. The question regarding relevance of this mechanism to humans remains a point of controversy [22].

In this same category of on-target pharmacologic mediated rodent carcinogenicity, human pathways associated with pharmacologic effects may diverge from pathways to neoplasms in rodents accounting for the lack of human relevance. Examples are provided here where human relevance is questionable under conditions of clinical use, taking into account the increased susceptibility of rodents to mechanisms of tumorigenesis, and the therapeutic margins relative to humans. Pathways for PPAR-alpha [11] and HMG-CoA reductase inhibitors [38] in humans appear to diverge from those in rodents.

Enterochromaffin-like cell tumors in male and female rats are a consequence of intended pharmacologic actions to increase stomach pH and the constant stimulation results in hypergastrinemia [2, 4]. Forestomach neoplasms in rodents are considered to be due to prolonged exposure of the drug to the forestomach in rodents. Humans do not have a forestomach, nor do they have Harderian or Zymbal glands. Therefore nongenotoxic mechanisms driving tumors specific to these organs would not be expected to be clinically relevant to humans.

Another example are alpha-glucosidase inhibitors result in a deprivation of colonic carbohydrate absorption, triggering a series of monitorable events leading to renal neoplasms in rats that can all be prevented by supplementation with glucose [24].

In the hematopoietic system, thymic lymphomas in mice that are secondary to a murine viral infection following immunosuppression may not be specifically relevant to humans. However, humans might experience other relevant effects resulting in the formation of tumors at other tissue sites from other infectious agents when the desired pharmacology of immune suppression is achieved, and effects are not necessarily a direct undesirable effect of a modifiable structure of the drug. The extent of effects depends on viral infection and viral load and not only on drug dose. Such findings of immunosuppressants in rodent studies may generally result in a labeled class warning.

## 7.4 Drug-Induced Rodent Tumors Can Be Associated with Off-Target and Secondary Pharmacology

In a second category are examples of drugs that result in rodent neoplasms due to off-target pharmacologic actions that are not a primary result of interaction of drug with the intended therapeutic targets. These off-target or secondary pharmacologic actions are often shown to be of questionable human relevance because many rodent hormonal pathways and hormonal levels are easier to perturb in rodents than in humans and as a result such disturbance over prolonged periods of drug administration will result in rodent tumors. F-Cell thyroid neoplasms in male rats can be

secondary to drug-related liver enzyme induction and drug-related decreases in T3 and T4 with associated increases in TSH [39]. This disturbance in the negative endocrine feedback loop results in sustained stimulation of thyroid F-cells by TSH, thyroid hyperplasia, and F-cell neoplasms in rats. However, sponsors have submitted data to regulatory authorities to support drug submissions showing that TSH was not increased in humans under conditions of clinical use. Leydig cell (interstitial cell) tumors are caused in rats by various drugs when testosterone levels are depleted and LH is increased [10, 12]. This has been shown to be prevented by testosterone supplementation in rats. Testosterone has been shown by sponsors for numerous drugs to not be depleted in humans under conditions of use.

Pathways for CAR agonists appear to diverge in humans from those in rodents [15, 51]. Epidemiology data have been generated for some drugs to support irrelevance of some rat liver neoplasms (e.g., phenobarbital [35]). Most hemangiosarcomas in mice from nongenotoxic drugs probably result from rodent specific pathways [9].

Renal neoplasms in male rats related to alpha-2-u-globulin nephropathy are concluded to be rat specific [48]. Urinary bladder neoplasms have also occurred in rats secondary to pharmacology, for example with PPAR dual alpha-gamma agonists. For some such agents the neoplastic effect appears dependent on drug induced secondary mechanisms resulting in altered urine composition, precipitation of salts of endogenous minerals, and enhanced urolithiasis irritating to the bladder wall [14], while for others [36] urinary bladder neoplasms are observed in the absence of any changes in urinary sediment or mineralization. However, urinary bladder neoplasms in rodents secondary to mineralization of drug substance in the bladder upon elimination of high doses has been seen with numerous agents and is considered to not be relevant to humans when no such crystals are seen in urine in humans [8].

Drug-induced liver neoplasms in mice and rats are usually irrelevant to humans, especially when secondary to liver toxicity, or associated with a high background rate in rodents. In general, neoplasms in rodent strains with a high background control rate, are often strain specific and not usually relevant to humans (e.g., pituitary neoplasms). In these cases rodents may be rather debilitated at the end of a 2 year study which can confound interpretation of the results. Furthermore, the chance occurrence and appearance of a drug associated increase in tumor rates must be carefully considered, and upper bounds of historical control tumor rates can be very helpful in this regard.

For non-DNA reactive drugs there is usually an exposure threshold for carcinogenicity below which there is little risk for humans. Neoplasms seen only at lethal doses are generally not considered relevant to humans but when the MTD that is exceeded results in exposures that are achieved at the human recommended dose the results may not be easily dismissed. Neoplasms seen at >25× the human exposure are generally not considered relevant to humans.

Many disease states are associated with alterations (increases or decreases) in normal physiology (e.g., continued immune activation, hyperglycemia). In humans, the intent of therapeutic intervention with a drug is to bring the disease state closer to normal. However, in carcinogenicity studies, normal animals are often exposed to doses of a drug that may cause sustained changes in physiology. An example is

adrenal pheochromocytomas in rats following treatment with certain SGLT2 inhibitors. These drugs cause glucose malabsorption due to off-target SGLT1 inhibition seen at the doses administered to rats, which in turn increases calcium absorption by stimulating colonic glucose fermentation and reducing intestinal pH. The resulting kidney tumors, pheochromocytomas and adrenal medullary hyperplasia seen in rats after lifetime exposure have been attributed to the sequelae of enhanced Ca++ intestinal absorption [13]. This does not happen in humans presenting initially with hyperglycemia and being given doses of SGLT-2 inhibitor which normalize blood glucose. Thus the neoplastic findings in normal rodents are not likely to be relevant to humans.

Another example is that of a drug with estrogenic activity and administered to animals with estrogen dominance in old age. Effects in animals will differ from those in humans deficient in estrogen. Uterine neoplasm development can be enhanced due to estrogen dominance in aged female rats. Somatostatin analogs, for example, can result in a high estrogen/progesterone ratio and a suppressed LH response to GnRH. This does not happen in humans.

## 7.5 Sponsors Are Expected to Provide Convincing Data Supporting a Conclusion That a New Test Agent Triggers the Same Key Events Critical to Driving the Same Mode of Action Previously Established Not to Be Human Relevant for Other Test Agents

It is important to note in the examples provided, that it may not be sufficient to simply point out to regulatory authorities that a rat carcinogenicity study with a new test agent yields tumors that resemble a pattern previously established not to be human relevant, such as thyroid follicular cell tumors seen in conjunction with liver hypertrophy. It is not uncommon for a new test agent discovered to cause thyroid follicular cell tumors in association with liver hypertrophy or liver tumors, but it would be important for a sponsor to show as well, that the test agent may also be a CAR activating enzyme inducer showing evidence of gene expression data, and also enhancement of thyroid hormone turnover. Data demonstrating these key events would provide confidence in the conclusion that the mode of action for the new test agent matches that of previous agents causing thyroid and liver tumors through the same key events and that the overall mode of action could be accepted and agreed to be human irrelevant. Such mode of action framework proposals have been summarized by Elcombe et al. [15] using phenobarbital as a prototypical CAR activating rodent liver carcinogen. Similar data have been described recently by Buckley et al. [6] for example for prasugrel reported to induce hepatocellular adenomas in mice that were considered secondary to enzyme induction and not relevant to human safety, and investigators [23] have proposed that liver cyp2b10 mRNA levels might be used as a biomarker of CAR activation to help address human irrelevance of

rodent liver tumor findings seen with dalcetrapib. More complete investigative approaches can be seen in freedom of information dossiers for numerous pharmaceuticals shown by sponsors to be CAR inducers, including recently approved darunavir (HIV protease inhibitor) and lorcaserin (serotonin 2C receptor agonist) both with labels indicating liver and thyroid tumors seen in rats attributed to hepatic enzyme induction with limited relevance to humans.

## 7.6 Is a 2-Year Mouse Carcinogenicity Study Still Needed?

Most carcinogenicity assessments for pharmaceuticals that are conducted in mice are now conducted in Tg.rasH2 mice except for drugs administered by dermal application. Inadequate data exist to determine if the Tg.rasH2 mouse model is appropriate for dermal application although future studies could address this issue. However, there are very few drug products applied dermally that have resulted in skin neoplasms in the past 20 years.

It has been pointed out recently [47] that one advantage of the 2-year mouse model over the Tg.rasH2 model is that a 25× human exposure threshold can be used to set the top dose while for the Tg.rasH2 model this is not presently acceptable regulatory practice, as pointed out above. For potent drugs dosed in humans at low exposures that are very well tolerated in mice, the doses and exposures that may be needed according to current ICHS1B guidance in a 6-month Tg.rasH2 mouse study would significantly exceed the doses deemed acceptable for a 2-year mouse study. Because results in a TgrasH2 study are not lifetime exposure and in possibly more susceptible animals than nontransgenic animals, they are considered to be valuable for hazard ID and not a more precise risk assessment tool. A compilation and review of accumulated experience and data is needed, to include a comparison of the relative drug exposures necessary to drive positive tumor outcomes for the same compounds conducted in both 2-year rat and 6-month TgrasH2 studies. Such data could support a systematic data-driven approach to establishing a reasonable exposure based threshold for setting doses in TgrasH2 mice, and thereby even further reduce the occasional need to conduct a 2-year mouse carcinogenicity study.

## 7.7 The Expanding Role of the Tg.rasH2 Alternative Transgenic Mouse Model

Since the ICH Expert Working Group on Safety introduced ICHS1B in 1996 [27], the door was opened for scientists to choose a short or medium-term rodent study as an alternative to one of the 2-year rodent carcinogenicity studies. This guideline stimulated international collaboration to evaluate the performance and utility of newly available transgenic mouse models for carcinogenicity testing, and the results of 4 years of research with the models have been summarized in a special issue of

**Table 7.1** Advantages to conducting a 6-month transgenic mouse assay

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| 1. Earlier insight to pharmaceutical carcinogenic potential |
| Enhanced overall clinical trial safety |
| Earlier re-direction of sponsors away from non-viable to more viable test candidates |
| Earlier resolution of hypothetical carcinogenicity concerns |
| Earlier trigger for investigative studies to understand cause for any human concern |
| 2. Can provide some mode-of-action understanding of positive findings |
| 3. May enable adoption of a strategy that eliminates the 2-year rat carcinogenicity testing timeline for clear noncarcinogens |
| 4. Reduction and refinements of animal use |
| 5. Significant savings in overall testing costs |
| Reduced test article demands |
| Animal husbandry costs for 6 month vs. 2 years of study activities |
| Histopathology assessment costs reduced with fewer animal numbers |
| Test facility space requirement demands are reduced and allow greater scheduling flexibility |
| 6. Reduced chance for a rodent-specific and human irrelevant false positive outcome |

Reprinted from [47]

*Toxicologic Pathology* [49]. In those early years prior to 2003, less than 25 % of carcinogenicity study protocols being proposed in the mouse to the USFDA were requesting an alternative short or medium term mouse model, and of the models proposed in those early years only a minority were for the Tg.rasH2 model [37]. Since then the popularity of the Tg.rasH2 model in particular has grown and in 2013 and 2014, approximately 75 % of all mouse carcinogenicity studies protocols for pharmaceutical development now propose the Tg.rasH2 model [33]. Among the models evaluated, the Tg.rasH2 was deemed most versatile in its superior ability over the other new models to detect relevant human carcinogens working through both genotoxic and nongentoxic mechanisms within 6 months of dosing. The model also was shown to improve on the poor specificity of the 2-year mouse assay by avoiding detection of numerous human irrelevant rodent carcinogens [44] and this conclusion has been confirmed in a recent analysis [40] of 21 publicly available Tg.rasH2 studies used to support pharmaceutical marketing registration and of all 38 studies received by the FDA by June 2014 [33]. The scientific, strategic and business advantages to industry for conducting a 6-month transgenic mouse study rather than the standard 2-year mouse assay have been summarized in Table 7.1 (Adapted from [47]).

Initial delay in the pace of adoption of the Tg.rasH2 model appears to have been based at least partially on the perceived risk that a single spontaneous tumor appearing in a high dose animal might raise concerns regarding test compound carcinogenic potential. Since the initial roll out of the model, historical control data for spontaneous tumor incidence have accumulated [41, 43] and based on the documented relatively low incidence in spontaneous tumors, except for splenic hemangiosarcomas and alveolar bronchiolar pulmonary neoplasms, and the growing trend in the use of the Tg.rasH2 model, the industry experience with this model appears to have alleviated these concerns.

## 7.8 Studies in Other Species

Carcinogenicity studies are not commonly performed in hamsters. However, in one recent case in which a carcinogenicity study was conducted in hamsters, hamsters had the pharmacologic activity when rats and mice didn't. In another recent case, the hamsters had a major human metabolite not seen in rats or mice.

## 7.9 Future Opportunities

As described, initiatives resulting in successful modifications to carcinogenicity testing over the past 20+ years, have been driven by supporting data and shared experience between drug regulatory authorities and pharmaceutical developers through ICH negotiation. The most recent initiative launched to modify ICH Carcinogenicity Testing Guidance seeks a logical risk-based approach to eliminate the need for 2-year rat study testing of those compounds with a recognizable strong safety profile, as well as for compounds where human relevant carcinogenicity risk is expected and no benefit would be gained from the conduct of a 2 year rat study. For compounds with a strong safety profile, the approach would be based on all test evidence accumulated indicating an absence of possible off-target effects including hormonal perturbation, genotoxicity and histologic evidence from chronic toxicology studies, along with knowledge of intended on-target pharmacology. For such compounds a transgenic mouse carcinogenicity study might suffice. In this way, resources for the conduct of 2-year rat studies could be reserved for compounds with signals from chronic studies or target pharmacology indicating uncertain risk, and both transgenic mouse and full 2-year rat carcinogenicity studies should be conducted. Furthermore, potentially informative endpoints should be incorporated early and proactively to inform understanding of key events and mode of action relating to human relevance. A concept paper and business case [7] were agreed upon by EMA, FDA, PMDA, EFPIA, JPMA and PhRMA, and an ICH SI Expert Working Group was launched. The business case is based on a published proposed decision paradigm suggested by PhRMA indicating that the outcome of past positive 2-year rat carcinogenicity studies with pharmaceutical candidates could be predicted with 80 % accuracy from information available from shorter term studies [46]. This analysis followed on the heels of an earlier study of data from 80 marketed pharmaceuticals demonstrating that the absence of evidence for preneoplastic potential in all tissues in chronic rat studies was a strong negative predictor of tumor outcome in any tissue [45]. The JPMA and FDA have each conducted independent analyses of separate databases that include an additional 60 and 50 pharmaceuticals, respectively, reaching the same conclusions. This further supports the notion that the number of 2-year rat studies could be reduced under certain conditions by approximately 40 % or more, without significant risk to the public health. Each 2-year rat study: (1) uses ~600 animals (2) adds 2–3 years for completion of

nonclinical studies supporting registration, and in so doing can in certain situations prolong the regulatory process and delay patient access to those new medications unless carcinogenicity studies are started at risk; (3) expends industry resources to plan, synthesize and formulate test article, conduct, analyze, report, and file (and also Regulatory Authority resources to review globally) – with a total cost of an estimated $3.75 M. The ICH S1 EWG was therefore convened, and a Regulatory Notice Document was agreed upon, drafted and posted [17] triggering a prospective test of the hypothesis. Additional supporting analyses for the current ICH initiative include an assessment of U.S. FDA drug labeling of carcinogenicity risk by Alden et al. [1] and an assessment of carcinogenicity studies for European pharmaceuticals approved for marketing between 1995 and 2009 by Friedrich and Olejniczak [18]. These authors separately concluded that carcinogenicity testing results often provided little value to the drug label that could not be otherwise obtained from an integration of shorter term study and test results.

The recently posted Regulatory Notice Document proposes that cancer risk of a new pharmaceutical can be predicted from data described above with sufficient certainty to be classified into one of three categories:

Category 1 – highly likely to be tumorigenic in humans such that a product would be labeled accordingly and 2-year rat, 2-year mouse, or transgenic mouse carcinogenicity studies would not add value.

Category 2 – the available sets of pharmacologic and toxicologic data indicate that tumorigenic potential for humans is uncertain and rodent carcinogenicity studies are likely to add value to human risk assessment. Accordingly, current S1B Guidance describes options for rodent carcinogenicity testing.

Category 3a – highly likely to be tumorigenic in rats but not in humans through prior established and well recognized mechanisms known to be rodent specific and human irrelevant, such that a 2-year rat study would not add value; or

Category 3b – highly likely not to be tumorigenic in both rats or humans such that no 2-year rat study is needed.

A prospective testing period was deemed necessary and agreed to by S1 EWG members to confirm that the same opportunities to exempt animal carcinogenicity testing are mutually visible and agreeable and accurately predictable to individual sponsors and to regulatory authorities in all three major ICH regions, before the outcomes of 2-year test results are known. Regulatory authorities will need to agree globally and practice a new process with clear criteria that involve an assessment of the adequacy and the interpretation of the data available from shorter term tests for exempting the conduct of a 2-year rat carcinogenicity study. The study data and relevant published literature expected to meet the criteria for submission of such a waiver request are described in detail as Appendix 1 in the posted Regulatory Notice Document [17] and summarized in Table 7.2. After gaining sufficient experience with processes and procedures for alignment on the new paradigm, and if outcomes are demonstrated to match predictions and expectations, then ICH members propose to adopt the new approach and modify current guidance accordingly. The accumulated actual results of approximately 2 years of

**Table 7.2** Weight of evidence to be considered for a categorical assignment in the CAD

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| 1. Knowledge of intended drug target and pathway pharmacology, secondary pharmacology, & drug target distribution in rats and humans |
| 2. Genetic toxicology study results |
| 3. Histopathologic evaluation of repeated dose rat toxicology studies with emphasis on chronic studies |
| 4. Exposure margins in chronic rat toxicology studies |
| 5. Metabolic profile |
| 6. Evidence of hormonal perturbation |
| 7. Immune suppression |
| 8. Special studies and endpoints |
| 9. Results of non-rodent chronic study |
| 10. Transgenic mouse study (not required for CAD prediction but can contribute if available) |

Adapted from Regulatory Notice Document [17]

accumulated study outcome predictions are expected to support guidance modifications by 2017/2018.

It has been pointed out recently that such a future framework involving waivers of 2-year rat studies would synergize especially well with the growing comfort in the use in particular of the Tg.rasH2 transgenic mouse model for evaluating human pharmaceutical carcinogenicity potential [40]. Waivers of 2-year rat carcinogenicity studies could lead to significant reductions in drug development timelines and shorter overall timelines to getting important new pharmaceuticals in the market to meet the medical needs of patients in those instances when the carcinogenicity evaluation can be fulfilled with early conduct of carcinogenicity studies in a single species using the 6-month Tg.rasH2 mouse. Continued use of the 2-year mouse in such circumstances would negate this advantage. In this regard, it will become important to further consider how agreement might be reached toward reasonable modification to the 25× exposure margin dose setting criteria currently limiting use of the Tg.rasH2 model for certain well tolerated pharmaceutical candidates, without introducing risk to human safety.

## 7.10 Conclusions

Experience collected over decades of a steadily evolving carcinogenicity testing paradigm, is steadily supporting a healthy dialog between regulatory authorities and drug sponsors as to what is necessary and sufficient to ensure human safety while being sensible with resources needed to conduct these very demanding studies. Genetically modified mice, especially the Tg.rasH2 model are becoming mainstays of pharmaceutical carcinogenicity testing, and creative investigative approaches and novel endpoints are wisely and increasingly being deployed to address questions regarding human relevance of positive rodent carcinogenicity study outcomes.

Finally, efforts have been launched recently through ICH to drive global alignment in a data driven manner toward guidance revisions that could further reduce the need for the 2-year rat study, allowing study conduct waivers when it makes sense, while maintaining the transgenic mouse for carcinogenicity testing. Before ICH guidance modifications involving waivers for the conduct of certain 2 years rat carcinogenicity studies can be considered, a prospective testing period has been launched engaging drug regulatory agencies in a world-wide collaboration with pharmaceutical sponsors to evaluate predictions of 2-year rat study outcomes on drugs in active development.

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