# S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals



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# FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner. By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

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INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

**ICH HARMONISED GUIDELINE**

# TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS
# S1B Addendum

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*At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.*

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## ICH HARMONISED GUIDELINE

# TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS

# ICH S1B ADDENDUM

### ICH Consensus Guideline

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## PREAMBLE

This Addendum is to be used in close conjunction with ICH *S1A Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals, S1B Testing for Carcinogenicity of Pharmaceuticals, and S1C(R2) Dose Selection for Carcinogenicity Studies*. The Addendum is complementary to the S1 Guidelines.

## 1. INTRODUCTION

### 1.1 Scope of the Addendum

This Addendum covers all small molecule pharmaceuticals where carcinogenicity evaluations are recommended as described in S1A.

### 1.2 Purpose of the Addendum

This Addendum expands the testing scheme for assessing human carcinogenic risk of small molecule pharmaceuticals by introducing an additional approach that is not described in the original S1B Guideline. This is an integrative approach that provides specific weight of evidence [WoE] criteria that inform whether or not a 2-year rat study adds value in completing a human carcinogenicity risk assessment. The Addendum also adds a plasma exposure ratio-based approach for setting the high dose in the rasH2-Tg mouse model,[1] while all other aspects of the recommendations for high dose selection in S1C(R2) Guideline would still apply.

Application of this integrative approach would reduce the use of animals in accordance with the 3Rs (reduce/refine/replace) principles, and shift resources to focus onto generating more scientific mechanism-based carcinogenicity assessments, while promoting safe and ethical development of new small molecule pharmaceuticals.

### 1.3 Background

While the S1B Guideline calls for flexibility in considering approaches to address pharmaceutical carcinogenicity testing, the basic scheme generally recommends a long-term rodent study which, in practice, is usually a 2-year study in rats, along with a second rodent carcinogenicity study in mice (2-year or short-term study). Since publication of the ICH S1B Guideline, scientific advances toward elucidation of mechanisms of tumorigenic action, greater understanding of the limitations of rodent models, and several retrospective analyses of pharmaceutical datasets indicate that 2-year rat carcinogenicity studies might not add value to human carcinogenicity risk assessment in some cases and the carcinogenic potential could have

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[1] The rasH2-Tg mouse was developed in the laboratory of Tatsuji Nomura of the Central Institute for Experimental Animals (1). The model is referred to in the S1B Guideline as the TgHras2 transgenic mouse. The official nomenclature for the model is CByB6F1-Tg(HRAS)2Jic which is maintained by intercrossing C57BL/6JJic-Tg(HRAS)2Jic hemizygous male mice with BALB/cByJJic female mice. The littermates derived from these intercrosses are the transgenic rasH2-Tg animals with the tg/wt genotype, and the wild type rasH2-Wt animals with a wt/wt genotype.
Since other short-term models mentioned in S1B have not gained significant use compared to rasH2-Tg over the past 20 years, pharmaceutical development experience with these models is far more limited. Therefore, other short-term carcinogenicity models referred to in S1B would not qualify for a plasma exposure ratio-based high dose selection.
It is appropriate to use wild-type rasH2-Wt littermates of rasH2-Tg mice for dose range-finding studies and for generating exposure data.

been assessed adequately based on a comprehensive assessment of all available pharmacological, biological, and toxicological data (2-9).

To determine whether the conclusions from these retrospective analyses could be confirmed in a real- world setting (i.e., prior to knowledge of the 2-year rat carcinogenicity study outcomes), an independent international prospective study was conducted under ICH *S1(R1) RND Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals – Regulatory Notice Document*. The conclusion from this prospective evaluation confirmed that an integrated WoE approach could be used to adequately assess the human carcinogenic risk for certain pharmaceuticals in lieu of conducting a 2-year rat study.[2]

In addition, an exposure ratio endpoint (based on animal to human plasma AUC) for high dose selection in 2-year rodent studies as per ICH S1C(R2) has not been globally accepted for use in the rasH2-Tg mouse study. Therefore, a comprehensive analysis was conducted to assess exposures and outcomes in rasH2-Tg studies from available information.[3] As described in Section 3, the results of this analysis indicate that there is no value in exceeding a 50-fold exposure ratio for high dose selection in this model.

## 2. A WEIGHT OF EVIDENCE APPROACH TO ASSESS THE HUMAN CARCINOGENIC POTENTIAL OF SMALL MOLECULE PHARMACEUTICALS

Over the course of drug development, it is important for sponsors to develop a scientifically robust strategy for carcinogenicity assessment that considers key biologic, pharmacologic, and toxicologic information. The integrative WoE assessment approach described in sections 2.1 and 2.2 may support a conclusion that the test compound is either:

- likely to be carcinogenic in humans such that the product would be labeled accordingly and any 2-year rat carcinogenicity studies would not add value; or
- likely not to be carcinogenic in humans such that a 2-year rat study would not add value (may also not be carcinogenic in rats, or may likely be carcinogenic in rats but through

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[2] Conduct and results of the prospective study will be summarized; ICH Website of RND and PEP updates will be cited; and future DRA manuscript pointed to. These new citations will appear in the Step 4 Version and this footnote modified.

[3] The approach taken for determining an adequate exposure margin for high dose selection for the rasH2-Tg short-term model is similar to that described previously for the 2-year rat and mouse studies (10,11) and Hisada S, Tsubota K, et al (Manuscript in preparation) Survey of Available Data to Assess Tumorigenic Sensitivity of rasH2-Tg Mice and 2-year Rodent Models. Draft Summary: Results were analyzed from studies conducted for 50 drugs in the 6-month rasH2-Tg model and the 2-year rat, 15 of which were also evaluated in the 2-year mouse. For 13 studies concluded to be positive in rasH2-Tg, 6 genotoxic carcinogens were positive within 0.1 - 3-fold of the AUC exposure ratio or body surface area adjusted dose ratio (rodent:human), and 7 nongenotoxic carcinogens were positive all within 1 - 50-fold. Among those 7, three tested positive only at exposures evaluated that exceeded 25-fold. The rasH2-Tg model was 20-fold more sensitive to 10-fold less sensitive than the 2-yr rat or mouse among these 13 drugs that were tested in all 3 models, while 3 of the 13 drugs tested negative in the 2-year rat study. Eight of 37 drugs that tested negative in rasH2-Tg were evaluated at greater than 50-fold exposure ratios (60 to >200-fold). For 11 compounds testing positive in 2-year rat studies at exposure ratios of <25-fold, and testing negative in rasH2-Tg, high dose selection in rasH2-Tg was limited by maximum tolerated dose (MTD) at exposure ratios of <50-fold for 9 drugs, and for the other 2 drugs, exposure margins exceeded 50-fold. Human relevance of the tumorigenic potential observed in rats for these 11 drugs has been questioned. In conclusion, when high exposures are tolerated in rasH2-Tg mice, there appears to be some value in exceeding 25-fold, but the overall evidence indicates no benefit to exceeding a 50-fold exposure margin. (Note: this summary paragraph may be deleted upon publication of Hisada et al).

well recognized mechanisms known to be human irrelevant); or

- uncertain with respect to the carcinogenic potential for humans, and a 2-year rat carcinogenicity study is likely to add value to human risk assessment.

In cases where the WoE assessment leads to a conclusion of uncertainty regarding human carcinogenicity potential, the approach described in S1B of conducting a 2-year rat carcinogenicity study together with a carcinogenicity assessment in mice (short term or 2-year study) remains the most appropriate strategy.

### 2.1 Factors to consider for a WoE assessment

A WoE approach is based on a comprehensive assessment of the totality of data relevant to carcinogenic potential available from public sources and from conventional drug development studies. These factors include:

1) data that inform carcinogenic potential based on drug target biology and the primary pharmacologic mechanism of the parent compound and active major human metabolites. This includes drug target distribution in rat and human; available information from genetically engineered models; human genetic association studies; cancer gene databases; and carcinogenicity information available on the drug class,
2) results from secondary pharmacology screens for the parent compound and major metabolites that inform off-target potential, especially those that inform carcinogenic risk (e.g., binding to nuclear receptors),
3) histopathology data from repeated-dose toxicity studies completed with the test agent, with particular emphasis on the long term rat study, including exposure margin assessments of parent drug and major metabolites,[4]
4) evidence for hormonal perturbation, including knowledge of drug target and compensatory endocrine response mechanisms; weight, gross and microscopic changes in endocrine and reproductive organs from repeated-dose toxicity studies; and results from reproductive toxicology studies,[5]
5) genetic toxicology study data using criteria from ICH S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use; equivocal genotoxicity increases uncertainty with respect to the carcinogenic potential,
6) evidence of immune modulation in accordance with ICH S8 Immunotoxicity Studies for Human Pharmaceuticals; it is generally recognized (12,13) that standard rat and mouse carcinogenicity studies are not reliable for identifying this specific human risk.

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[4] Histopathology findings from long term rat toxicity studies of particular interest for identifying carcinogenic potential in a 2-year rat study include cellular hypertrophy, cellular hyperplasia, persistent tissue injury and/or chronic inflammation, foci of cellular alteration, preneoplastic changes, and tumors. It is important to provide an understanding of the likely pathogenesis, and/or address the human relevance of such findings. While long term rat toxicity study data are shown to be of highest value for assessing the likely outcome and value of conducting a 2-year rat study, short term rat studies can sometimes also provide histopathologic conclusions of value.
Data from long term toxicity studies in non-rodents and mice may also be useful for providing additional context on the human relevance of rat study findings (e.g., species-specific mechanistic differences) and whether there is value in conducting a 2-yr rat study.

[5] If microscopic changes in endocrine and reproductive tissues including atrophy, hypertrophy, hyperplasia are observed, or statistically and biologically significant test article associated endocrine or reproductive organ weight changes are observed this may be considered evidence of functional hormonal perturbation even when changes in hormone levels are not documented. Such findings may be suggestive of potential carcinogenic risk unless investigated for human relevance and demonstrated otherwise.

The above WoE factors may be sufficient to conclude whether or not a 2-year rat study would add value. However, where one or more WoE factors may be inconclusive or indicate a concern for carcinogenicity, the Sponsor can conduct investigations that could inform human relevance of the potential risk. Possible approaches may include, but are not limited to:

1) additional investigational studies, or analyses of specimens collected from prior studies (e.g., special histochemical stains, molecular biomarkers, serum hormone levels, further characterization of immunomodulation, alternative *in vitro* or *in vivo* test systems, data from emerging technologies, etc.), and
2) clinical data generated to inform human mechanistic relevance at therapeutic doses and exposures (e.g., urine drug concentrations and evidence of crystal formation; targeted measurements of clinical plasma hormonal alterations; human imaging data, etc.).

### 2.2 Integration of WoE Factors for Assessing Human Carcinogenic Risk

An integrated analysis of the WoE factors described above determines whether or not a standard 2-year rat study would contribute to the human carcinogenic risk assessment. While all factors will contribute to the integrated analysis, the relative importance of each factor will vary depending on the specific molecule being considered. A summary of key outcomes and examples based on the experience accrued during the ICH S1 RND study (*S1(R1) RND Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals – Regulatory Notice Document*), are provided in Appendix 1 demonstrating how the WoE factors could be integrated in determining the need for a 2-year rat study.

Experience from the ICH S1 RND study indicates that an established profile of other compound(s) in a drug class contributes substantially to assessing human carcinogenic risk associated with modulation of the pharmacologic target. Compounds with novel drug targets (i.e., first-in-class) are, nevertheless, considered eligible for an integrative WoE-based approach. For such candidates, a higher evidentiary standard is expected to establish that there is no cause-for-concern in regard to target biology. Appendix 1 provides an example where a WoE assessment led to a conclusion that a 2-year rat study would not add value to human carcinogenic risk assessment for a drug inhibiting a novel target.

When the WoE assessment concludes that conduct of a 2-year rat study is not warranted, the Sponsor should seek alignment with the Drug Regulatory Agency [DRA] of each region where marketing approval is sought. When a sponsor decides to conduct a 2-year rat study in accordance with ICH S1B, there is no obligation to seek concurrence nor to document their rationale with each DRA.

### 2.3 Mouse Carcinogenicity Studies

A carcinogenicity study in mice, either 2-year or a short-term transgenic model as specified in ICH S1B, remains a recommended component of a carcinogenicity assessment plan, even for those compounds where the integrated WoE assessment indicates a 2-year rat study would not contribute significant value.[6] However, in some cases, for example, when the WoE evaluation

[6] The WoE approach described for the rat is not appropriate for eliminating the mouse as a second rodent carcinogenicity species because: (1) 6-month chronic toxicity studies are not generally conducted with mice so the WoE approach cannot be implemented and no database is available to confirm this approach, (2) the results of carcinogenicity studies in mice will often provide different outcomes from the corresponding rat carcinogenicity study, so a direct extrapolation cannot be made, and (3) a 6-month rasH2-Tg mouse has been adopted as an acceptable carcinogenicity study model.
When the WoE evaluation indicates the 2-year rat study adds no value, a carcinogenicity study in mice (either 2-year or short-term) is also not recommended in the EU.

strongly indicates no carcinogenic risk to humans and data indicate that only subtherapeutic, pharmacologically inactive drug exposures can be achieved in the mouse, it may not be appropriate to conduct any mouse carcinogenicity study.

## 3. CLARIFICATION ON CRITERIA FOR SELECTION OF THE HIGH DOSE FOR RASH2-TG MOUSE CARCINOGENICITY STUDIES

In practice, a plasma exposure (AUC) ratio for high dose selection in the absence of dose limiting toxicity or appropriate use of other dose setting criteria as outlined in ICH S1C(R2) in this model, has not been globally accepted as an endpoint.[1] Therefore, available data from experience with 50 compounds evaluated in the rasH2-Tg mouse model were analyzed and the conclusion reached that there was no value in exceeding a 50-fold plasma AUC exposure ratio (rodent:human) to support carcinogenicity assessment. Therefore, all criteria for selection of the high dose for carcinogenicity studies as specified in S1C(R2) for 2-year rodent studies are applicable to rasH2-Tg, including an AUC plasma exposure ratio, except that the exposure ratio will be 50-fold in rasH2-Tg rather than 25-fold as for 2-year studies conducted in wild type rodents. All other aspects of S1C(R2) remain applicable to rasH2-Tg.

## REFERENCES


(1) Saitoh A, Kimura M, Takahashi R, Yokoyama M, Nomura T, Izawa M et al. Most tumors in transgenic mice with human c-Ha-ras gene contained somatically activated transgenes. Oncogene 1990;5(8):1195-200.

(2) Van Oosterhout JPJ, Van der Laan JW, De Waal EJ, Olejniczak K, Hilgenfeld M, Schmidt V et al. The utility of two rodent species in carcinogenic risk assessment of pharmaceuticals in Europe. Reg Toxicol Pharmacol 1997;25:6-17.

(3) Contrera JF, Jacobs AC, DeGeorge JJ. Carcinogenicity testing and the evaluation of regulatory requirements for pharmaceuticals. Reg Toxicol Pharmacol 1997;25:130-45.

(4) Reddy MV, Sistare FD, Christensen JS, DeLuca JG, Wollenberg GK, DeGeorge JJ. An evaluation of chronic 6- and 12-month rat toxicology studies as predictors of 2-year tumor outcome. Vet Pathol 2010;47:614–29.

(5) Sistare FD, Morton D, Alden C, Christensen J, Keller D, De Jonghe S et al. An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: support for a proposal to modify current regulatory guidelines. Toxicol Pathol 2011;39:716-44.

(6) Alden CL, Lynn A, Bourdeau A, Morton D, Sistare FD, Kadambi VJ et al. A critical review of the effectiveness of rodent pharmaceutical carcinogenesis testing in predicting for human risk. Vet Pathol 2011;48:772-84.

(7) Friedrich A, Olejniczak K. Evaluation of carcinogenicity studies of medicinal products for human use authorised via the European centralised procedure (1995-2009). Reg Toxicol Pharmacol 2011;60:225-48.

(8) Van der Laan JW, Kasper P, Lima BS, Jones DR, Pasanen M. Critical analysis of carcinogenicity study outcomes. Relationship with pharmacological properties. Crit Rev Toxicol 2016;46:587-614.

(9) Van der Laan JW, Buitenhuis WHW, Wagenaar L, Soffers AEMF, Van Someren EP, Krul CAM et al. Prediction of the carcinogenic potential of human pharmaceuticals using repeated dose toxicity data and their pharmacological properties. Frontiers in Medicine 2016;3:45. doi: 10.3389/fmed2016.00045

(10) Contrera JF, Jacobs AC, Prasanna HR, Mehta M, Schmidt WJ, De George J. A systemic exposure-based alternative to the maximum tolerated dose for carcinogenicity studies of human therapeutics. J Am Coll Toxicol 1995;14:1–10.

(11) Bergman K, Olofsson IM, Sjöberg P. Dose selection for carcinogenicity studies of pharmaceuticals: systemic exposure to phenacetin at carcinogenic dosage in the rat. Reg Toxicol Pharmacol 1998;28:226–29.

(12) Bugelski PJ, Volk A, Walker MA, Krayer JH, Martin P, Descotes J. Critical review of preclinical approaches to evaluate the potential of immunosuppressive drugs to influence human neoplasia. Int J Toxicol 2010;29:435-66.

(13) Lebrec H, Brennan FR, Haggerty H, Herzyk D, Kamperschroer C, Maier CC et al. HESI/FDA workshop on immunomodulators and cancer risk assessment: Building blocks for a weight-of-evidence approach. Reg Toxicol Pharmacol 2016;75: 72-80.

## APPENDIX 1: CASE STUDIES APPLYING THE WEIGHT OF EVIDENCE APPROACH

### Preamble

One outcome of the ICH S1 RND study was the recognition that programs with the following WoE attributes are more likely to support a conclusion that the results of a 2-year rat study would not contribute value to human carcinogenicity risk assessment.

- Target biology is well characterized and not associated with cellular pathways known to be involved with human cancer development. Often, the pharmaceutical target was non-mammalian and carcinogenicity data were available with the pharmacologic drug class.
- Results from chronic toxicity studies indicate no hyperplastic, hypertrophic, atypical cellular alterations, or degenerative/regenerative changes noted without adequate explanation of pathogenesis or human relevance, indicative of no on- or off-target potential of carcinogenic concern;
- No perturbation of endocrine and reproductive organs observed, or endocrine findings adequately explained with respect to potential human relevance;
- No identified concerns from secondary pharmacology screens intended to inform off-target potential for the pharmaceutical
- No evidence of immune modulation or immunotoxicity based on target biology and repeat dose toxicology studies
- The overall assessment of genotoxic potential is concluded to be negative based on criteria from ICH S2(R1) Guidance.

Although rasH2-Tg mouse study results were recommended when available as a WoE element in the initial RND, they did not significantly contribute to the prediction of the 2-year rat carcinogenicity study outcome. Therefore, a rasH2-Tg mouse study is not expected to be completed to support a WoE assessment. However, if rasH2-Tg mouse study results are available, they should be discussed in the assessment.

A series of case studies are provided to illustrate the application of the WoE approach. These cases are provided for illustrative purposes only and are not intended as guidance to indicate the sufficiency of data to support a WoE assessment. Cases 1 and 2 describe the key WoE factors for that pharmaceutical and how the data were integrated to conclude that a 2-year rat study would not add value to the assessment of carcinogenic risk. In contrast to these cases, Case 3 describes how data from the WoE factors were integrated to conclude that the carcinogenic potential for humans was uncertain, and a 2-year rat carcinogenicity study was likely to add value to human risk assessment.  Case 4 describes a molecule for which a 2-year rat carcinogenicity study was concluded to not contribute value to human carcinogenicity assessment despite there being no data available for other molecules within the pharmacologic class.

### Case 1: A small molecule inhibitor against a non-mammalian target

**Prospective WoE Assessment: Concluded by all DRAs and Sponsor as likely not to be carcinogenic in both rats or humans such that a 2-year rat study would not add value**

**Rationale**

The WoE analysis supports the conclusion that the molecule was sufficiently studied at high exposure margins, and cause-for-concern was not identified for any of the WoE factors.

**2-year Rat Study Results: No test article related neoplastic findings were present in the 2-year rat study**.

**WoE Criteria**

Knowledge of intended drug target and pathway pharmacology relative to carcinogenesis

- Non-mammalian target excludes intentional alteration of potential mammalian carcinogenic pathways.
- No evidence of carcinogenic outcome in 2-year rat studies conducted with other compounds with the same non-mammalian pharmacological target

Secondary Pharmacology Screen

- No evidence of off-target interactions at drug concentrations up to 10 µM, including no interaction with estrogen, androgen, glucocorticoid receptors

General Toxicology from Chronic Rat Study

- Chronic (6-month) toxicology study in Wistar rats dosed to saturation of absorption, achieving up to a 31-fold margin to human exposure.
- No evidence of human specific major metabolites.
- No treatment-related histopathologic findings observed in standard battery of tissues

General Toxicology from Chronic Non-rodent Study

- Chronic administration (9-month) to non-human primates identified bile duct hyperplasia and hepatocellular hypertrophy, with reactive neutrophils and regenerative hyperplasia. A No-Adverse-Effect-Level was identified which provided a 5-fold margin to human exposure.
- Further evaluation in rats would not provide useful information, as similar findings were not observed in the chronic rat study.

Hormonal Perturbation

- No treatment-related findings on reproductive organ weights or histopathology

Genetic Toxicology

- No evidence of genotoxic potential based on criteria from ICH S2(R1) Guidance

Immune Toxicology

- No treatment-related changes in clinical pathology or histopathology of immune tissues (e.g., lymphoid organs, spleen, thymus, bone marrow)

Additional Special Investigations

- No data available

## Case 2: A small molecule antagonist of a neuronal G-protein coupled receptor

**Prospective WoE Assessment: Unanimously concluded as likely to be carcinogenic in rats but not in humans through well recognized mechanisms known to be human irrelevant, such that a 2-year rat study would not add value**

**Rationale**
The WoE analysis indicates the potential for rodent-specific liver and thyroid neoplasms based on the toxicology observed in the chronic rat study and on tumor outcome with the pharmacological class. Induction of hepatic cytochrome P450 was demonstrated. Evidence of hormonal perturbation is understood from target pharmacology, did not result in changes in reproductive organ weight or histopathology, and occurred at high multiples to human exposure.

**2-year Rat Study Results: The 2-year rat study demonstrated hepatocellular hypertrophy but no neoplastic findings.**

**WoE Criteria**

Knowledge of intended drug target and pathway pharmacology relative to carcinogenesis

- Predominate receptor expression in brain with lower expression in some peripheral tissues, similar across species
- Receptor activation increases ACTH release from pituitary secondary to hypothalamic production of adrenocorticotropin-releasing hormone.
- Hypothalamic receptor ligand levels associated with LH surge and gonadotropin release in rats.
- Target knock-out mice showed no findings related to carcinogenicity.
- Long-term studies with other compound with same pharmacological target associated with thyroid follicular cell adenoma/carcinoma in rats, consistent with elevated thyroid stimulating hormone following off-target cytochrome P450 induction.
- Antagonist binding interaction identified for one off-target receptor with Ki 8-fold higher than Cmax at maximum clinical dose. Known target pharmacology of off-target receptor not associated with tumorigenesis.

General Toxicology from Chronic Rat Study

- Increased liver hypertrophy and organ weight at 50x to 74x margin to human exposure.
- Increased thyroid follicular hypertrophy at 170x to 670x margin to human exposure.
- No evidence of human specific metabolites.
- An active major human metabolite in humans was also present in rats

General Toxicology from Chronic Non-rodent Study

- Increased liver hypertrophy and organ weight at ~230-fold human exposure.

Hormonal Perturbation

- Reduced adrenal weight without histopathological correlates and reduced ACTH level at >74x human exposure in the chronic rat study, consistent with inhibition of drug target. Response noted to be growth suppressive.
- Irregular estrous cycles and decreased pregnancy rate were observed at 60-fold human exposure, and decreased numbers of corpora lutea, implantations, and live embryos were observed at >500-fold human exposure in a fertility study in rats. Considered consistent with inhibition of drug target.
- No treatment-related changes observed in reproductive organ weight or histopathology in chronic rat study.

Genetic Toxicology

- No evidence of genotoxic potential of parent or major human metabolite based on criteria from ICH S2(R1) Guidance

Immune Toxicology

- No treatment-related changes in clinical pathology, lymphocyte subsets, or histopathology of immune tissues (e.g., lymphoid organs, spleen, thymus, bone marrow)

Additional Special Investigations

- Increased induction of CYP1A2 and CYP3A1 demonstrated
- Bone and teeth fluorosis related to defluorination of compound, demonstrated not to occur in humans

**Case 3: A first-in-class small molecule inhibitor of a ubiquitously expressed serine/threonine kinase**

**Prospective WoE Assessment: Unanimously concluded to be uncertain with respect to the carcinogenic potential for humans, and a 2-year rat carcinogenicity study is likely to add value to human carcinogenicity assessment**

**Rationale**

Significant carcinogenic uncertainty is based on a complex target pharmacology, the lack of precedent with the drug target, and histopathological changes of concern with inadequate mechanistic explanation from the chronic rat study which are supported by similar findings in cynomolgus monkeys. The immune toxicology observed in monkey will contribute to the overall assessment of risk but is not expected to be further informed by a rat carcinogenicity study.

**2-year Rat Study Results: The 2-year rat study demonstrated an increased incidence, lethality, and reduced latency of pituitary tumors in both sexes. This carcinogenic outcome in rats would contribute to the overall assessment of human carcinogenic potential.**

**WoE Criteria**

Knowledge of intended drug target and pathway pharmacology relative to carcinogenesis

- Target activation by inflammation-related oxidative stress promotes cellular apoptosis and is linked to control of cell proliferation; target inhibition suppresses apoptotic signaling and impacts cell proliferation, theoretically promoting cancer growth.
- Drug target displays tissue-dependent roles in cancer development, both promotion and suppression, in animal models.
- No data available on tumor outcome from target inhibition in long term rodent or short term transgenic mouse studies

General Toxicology from Chronic Rat Study

- Increased incidence and severity of renal basophilic tubules, eosinophilic droplets, and brown pigment in renal cortex starting at 14-fold human exposure. Etiology of lesions not empirically addressed.

- Chronic irritation of limiting ridge in non-glandular stomach at 39-fold human exposure. Etiology of lesions not empirically addressed.
- Increased liver weight without microscopic correlates.
- No evidence of human specific metabolites.
- An inactive major human metabolite in humans was also present in rats

General Toxicology from Chronic Non-rodent Study

- In monkeys, gastrointestinal epithelial degeneration, necrosis, reactive hyperplasia, ectasia, inflammation, and ulceration, at doses ~12-fold human exposure
- Increased incidence of renal tubule degeneration /regeneration, necrosis, dilation, and vacuolation at ~12-fold human exposure

Hormonal Perturbation

- Increased adrenal weight and cortical hypertrophy in rats at 17-fold human exposure. Etiology not empirically addressed.

Immune Toxicology

- In monkeys, suppression of TDAR with no effect on NK cytotoxicity or granulocyte function, and decreased lymphoid cellularity in spleen, thymus, lymph nodes at 12-fold human exposure.

Genetic Toxicology

- No evidence of genotoxic potential of parent or major human metabolite based on criteria from ICH S2(R1) Guidance

Additional Special investigations

- Increases in hepatic enzymes CYPs 1A, 3A, and 2B demonstrated.

### Case 4: A first-in-class small molecule inhibitor of a prostaglandin receptor

**Prospective WoE Assessment: Unanimously concluded as likely not to be carcinogenic in both rats or humans such that a 2-year rat study would not add value**

**Rationale**

When compared with the test agent discussed in Case 3, which is also first-in-class, the drug target in Case 4 is not associated with a role in cancer development, histopathological findings were not observed in the chronic rat study, and a large margin of exposure was calculated at the high dose (>50x). The secondary pharmacology screen also indicated the test agent demonstrates target selectivity.

**2-year Rat Study Results: The 2-year rat carcinogenicity study did not demonstrate a dose-related increase in tumors.**

**WoE Criteria**

Knowledge of intended drug target biology and pharmacologic mechanism relative to carcinogenesis

- Receptor activation associated with allergic inflammatory response and currently available data do not suggest a role in tumor initiation or progression.
- Knock-out mice of drug target showed no histological abnormalities or effects on immune function during one year of observation.
- No data available on tumor outcome in 2-year rat studies conducted with other compounds with the same pharmacological target.
- No data available from a rasH2-Tg carcinogenicity study conducted with the test agent.

Secondary pharmacology screen

- Test agent was at least 300-fold more selective for drug target when compared with other receptors in the same class as well as a sub-set of other assessed receptors involved in the inflammatory response.
- Test agent was at least 2000-fold more selective for the drug target in a secondary pharmacology screen of various receptors, ion channels, transporters and enzymes.

General Toxicology from Chronic Rat Study

- Histopathological assessments conducted as part of repeated-dose toxicity studies up to 26-weeks indicated no proliferative changes in any organ or tissue at the highest dose tested (~ 54-fold human exposure based on AUC).
- No evidence of human specific metabolites.

General Toxicology from Chronic Non-rodent Study

- Histopathological assessments conducted as part of repeated-dose toxicity studies up to 39-weeks indicated no proliferative changes in any organ or tissue at the highest dose tested (~ 45-fold human exposure based on AUC).

Hormonal Perturbation

- No treatment-related findings on reproductive organ weights or histopathology.

Genetic Toxicology

- No evidence of genotoxic potential based on criteria from ICH S2(R1) Guidance.

Immune Toxicology

- In the 26-week rat toxicity study, there were no effects on immune function (including the TDAR assay evaluating primary and secondary antibody responses) or adverse effects on lymphocyte subsets at the highest dose tested (~54-fold human exposure based on AUC).

Additional Special Investigations

- Not performed.