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

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

November 2022
ICH

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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.

ICH is a consensus-driven process that involves technical experts from regulatory authorities and industry parties in detailed technical and science-based harmonization work that results in the development of ICH guidelines. The commitment to consistent adoption of these consensus-based guidelines by regulators around the globe is critical to realizing the benefits of safe, effective, and high-quality medicines for patients as well as for industry. As a Founding Regulatory Member of ICH, the Food and Drug Administration (FDA) plays a major role in the development of each of the ICH guidelines, which FDA then adopts and issues as guidance to industry.

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Contains Nonbinding Recommendations

S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals
Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

PREAMBLE

This Addendum is to be used in close conjunction with the ICH guidances for industry *S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals*[2] (March 1996), *S1B Testing for Carcinogenicity of Pharmaceuticals* (July 1997), and *S1C(R2) Dose Selection for Carcinogenicity Studies* (September 2008). The Addendum is complementary to the ICH S1 guidances for industry.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

I. INTRODUCTION (1)[3]

A. Scope of the Addendum (1.1)

This Addendum applies to all pharmaceuticals that need carcinogenicity testing as described in ICH S1A. For biotechnology-derived pharmaceuticals, refer to the ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (May 2012)).

[1] This guidance was developed within the Expert Working Group (Safety) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Assembly at *Step 4* of the ICH process, August 2022. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions.

[2] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[3] The numbers in parentheses reflect the organizational breakdown of the document endorsed by the ICH Steering Committee at *Step 4* of the ICH process, August 2022.

B. Purpose of the Addendum (1.2)

This Addendum expands the evaluation process for assessing human carcinogenic risk of pharmaceuticals by introducing an additional approach that is not described in the original ICH S1B. This is an integrative approach that provides specific weight of evidence (WoE) criteria that inform whether a 2-year rat study is likely to add value to 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,[4] while all other aspects of the recommendations for high-dose selection in ICH S1C(R2) still apply.

Application of this integrative approach reduces the use of animals in accordance with the 3R (reduce/refine/replace) principles and shifts resources to focus on generating more scientific mechanism-based carcinogenicity assessments, while continuing to promote safe and ethical development of new pharmaceuticals.

C. Background (1.3)

While ICH S1B calls for flexibility in considering approaches to address pharmaceutical carcinogenicity testing, the basic paradigm 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 ICH S1B, scientific advances toward elucidation of mechanisms of carcinogenicity, 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 been assessed adequately based on a comprehensive assessment of all available pharmacological, biological, and toxicological data (Refs. 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), a subsequent international prospective study was conducted under ICH S1(R1) *Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals – Regulatory Notice Document*[5]. The process and several status updates reporting results are posted and available at the ICH website (Refs. 10-14). Carcinogenicity assessment documents and associated data from 2-year rat carcinogenicity studies for 45 compounds were received and evaluated by regulatory members of the ICH Expert

[4] The rasH2-Tg mouse was developed in the laboratory of Tatsuji Nomura of the Central Institute for Experimental Animals (Ref. 1). The model is referred to in ICH S1B 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 mice with the tg/wt genotype, and the wild type rasH2-Wt mice with a wt/wt genotype. Since other short-term models mentioned in ICH S1B have not gained significant use compared to rasH2-Tg mouse over the past 20 years, pharmaceutical development experience with these models is far more limited. Therefore, other short-term carcinogenicity models referred to in ICH 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.

[5] Available at https://database.ich.org/sites/default/files/S1%28R1%29_EWG_RND.pdf

Working Group. 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.[6]

In addition, an exposure ratio endpoint based on animal to human plasma Area Under the Curve (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 mouse studies from available information (Ref. 15). As described in section 3, the results of this analysis indicate that a 50-fold plasma AUC exposure ratio (rodent:human) is an adequate criterion for high-dose selection.

II. A WEIGHT OF EVIDENCE APPROACH TO ASSESS THE HUMAN CARCINOGENIC POTENTIAL OF PHARMACEUTICALS (2)

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 carcinogenic potential of the pharmaceutical in humans is:

- Likely, such that a 2-year rat carcinogenicity study would not add value
- Unlikely, such that a 2-year rat carcinogenicity study would not add value[7]
- Uncertain, such that a 2-year rat carcinogenicity study would 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 long-term carcinogenicity study together with an additional in vivo carcinogenicity study remains the most appropriate strategy (Figure 1).

[6] Methods and results of the ICH S1 prospective evaluation study will be summarized in a future publication.
[7] A WoE assessment may indicate that a compound is likely to be carcinogenic in rats. The compound may not be considered carcinogenic in humans if there is sufficient evidence that the mechanism of carcinogenicity is irrelevant to humans.

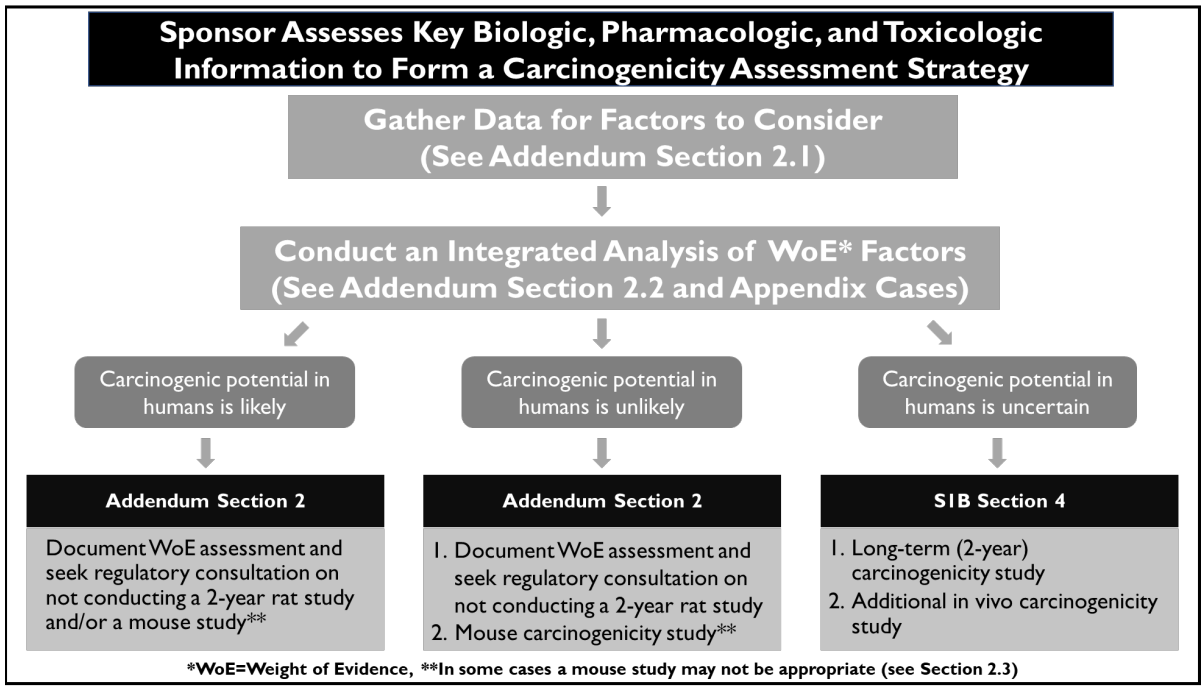

Figure 1: Flow scheme outlining key steps and options in developing a carcinogenicity assessment strategy and determining the added value of a 2-year rat study. Note that key biologic, pharmacologic, and toxicologic information should be assessed even when taking the ICH S1B approach that utilizes a 2-year rat study. When a sponsor decides to conduct a 2-year rat study in accordance with ICH S1B, there is no obligation to seek concurrence with the Drug Regulatory Agency (DRA). Refer to sections 2.1 and 2.2 for additional detail.

A. Factors to Consider for a WoE Assessment (2.1)

A WoE approach is based on a comprehensive assessment of the totality of data relevant to carcinogenic potential available from public sources and from relevant drug development studies. These factors include, but are not limited to:

(1) Data that inform carcinogenic potential based on drug target biology and the primary pharmacologic mechanism of the parent compound and major human metabolites; this includes drug target distribution in rats and humans along with the pharmacologic activity and potency of the parent compound and major metabolites in these species; available information from genetically engineered models; human genetic association studies; cancer gene databases; and carcinogenicity information on class effects, if available.

(2) Results from secondary pharmacology screens for the parent compound and major metabolites that inform selectivity and off-target potential, especially those that inform carcinogenic risk (e.g., binding to nuclear receptors) histopathology data[8] from repeated-dose toxicity

[8] Histopathology findings from 6-month 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

studies completed with the compound, with particular emphasis on the 6-month rat study, including plasma exposure margin assessments of parent drug and major metabolites.

(3) Evidence for hormonal perturbation,[9] 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 relevant results from reproductive toxicology studies, if available.

(4) Genetic toxicology study data using criteria from ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012); equivocal genotoxicity data that cannot be resolved in accordance with ICH S2(R1) recommendations increases uncertainty with respect to the carcinogenic potential

(5) Evidence of immune modulation in accordance with ICH S8. Evidence of broad immunosuppression may provide sufficient concern for human risk that would not be further informed by standard rat and mouse carcinogenicity studies (Refs. 16 and 17).

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

(1) Additional investigative studies or analyses of specimens collected from prior studies (e.g., special histochemical stains, molecular biomarkers, serum hormone levels, immune cell function, in vitro or in vivo test systems, data from emerging technologies)

(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)

A rasH2-Tg mouse study is not expected to be completed to support a WoE assessment.

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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 the 6-month rat toxicity study is the primary study to be used for assessing the likely outcome and value of conducting a 2-year rat study, shorter-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-year rat study.

[9] Findings from rat toxicity studies suggesting hormonal perturbation may include microscopic changes in endocrine or reproductive tissues of atrophy, hypertrophy, and hyperplasia and/or biologically significant endocrine and reproductive organ weight changes which are not explained as findings secondary to processes such as stress or altered body weight. Changes of this nature 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.

However, if rasH2-Tg mouse study results are available, they should be included in the WoE document.

B. Integration of WoE Factors for Assessing Human Carcinogenic Risk (2.2)

An integrated analysis of the WoE factors described above should be used to determine whether a 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 compound being considered (Figure 2).

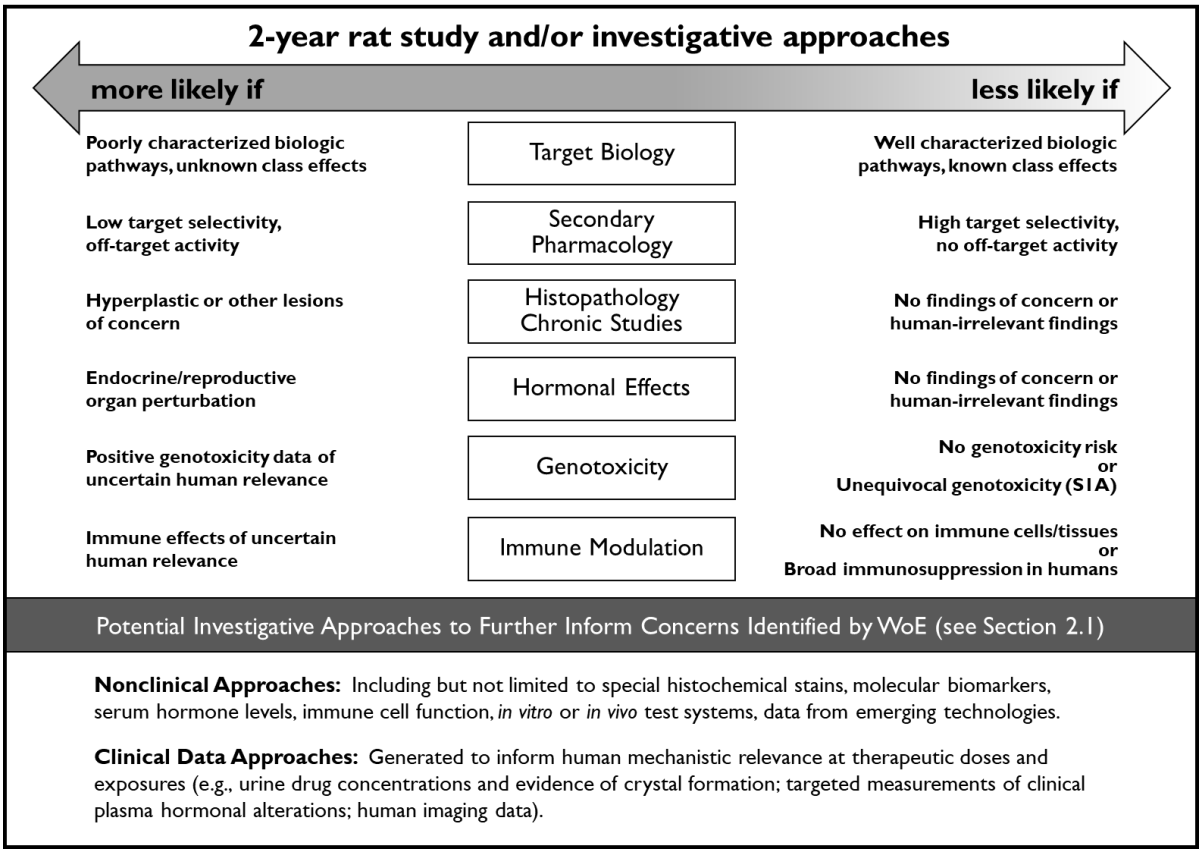

Figure 2: Integration of key WoE factors and potential investigative approaches to further inform on the value of conducting a 2-year rat study for assessment of human carcinogenic risk. When all WoE attributes align towards the right side of the figure, a conclusion that a 2-year rat study would not add value is more likely. Note that for the genotoxicity WoE factor a 2-year rat study is less likely to be of value either in cases where there is no genotoxicity risk or in cases with unequivocal genotoxicity risk. Similarly, for the immune modulation WoE factor, a 2-year rat study is less likely to be of value in cases where there are either no effects on the immune system or in cases where there is broad immunosuppression.

A summary of key outcomes and examples based on the experience accrued during the ICH S1 study see S1(R1) *Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals – Regulatory Notice Document*) are provided in the Appendix, demonstrating how the WoE factors

could be integrated to determine the value of conducting a 2-year rat study for assessment of human carcinogenic risk.

Experience from the ICH S1 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 assessment. For such compounds, further evidence that there is no cause-for-concern regarding target biology is needed to compensate for the lack of precedent. Case 4 in the Appendix describes an example for a novel target where a 2-year rat study was not considered to add value given sufficient evidence to compensate for the lack of precedent. In this example, a cause-for-carcinogenic-concern was not identified regarding drug target biology or compound selectivity, and no proliferative changes in any organs or tissues were observed at a high multiple of exposure in the 6-month study in rats (a pharmacologically relevant species).

When the WoE assessment supports a conclusion that conduct of a 2-year rat study does not add value to the assessment of human carcinogenic risk, the sponsor should seek consultation with the applicable DRA in accordance with the established regulatory consultation procedure for that region. When a sponsor decides to conduct a 2-year rat study in accordance with ICH S1B, there is no obligation to seek consultation with the DRA.

C. Mouse Carcinogenicity Studies (2.3)

A carcinogenicity study in mice, either a 2-year study in a standard strain of mice or a short-term study in a transgenic model as in ICH S1B, remains a recommended component of a carcinogenicity assessment plan, even for those compounds for which the WoE assessment indicates a 2-year rat study would not contribute significant value. Use of a transgenic model is consistent with the 3R (reduce/refine/replace) principles and this model should be prioritized unless there is a scientific rationale for conducting a 2-year study in mice.
There are cases where it may not be appropriate to conduct a mouse carcinogenicity study. As one example, a mouse study may not be appropriate when the WoE evaluation strongly indicates no carcinogenic risk to humans and the data indicate that only subtherapeutic and pharmacologically inactive drug levels relative to human exposure can be achieved in the mouse. As an additional example, when the WoE assessment indicates that a compound is likely to be carcinogenic in humans, the conduct of a mouse study may not be appropriate.

III. CLARIFICATION ON CRITERIA FOR HIGH-DOSE SELECTION BASED ON EXPOSURE FOR RASH2-TG MOUSE CARCINOGENICITY STUDIES (3)

A plasma exposure (AUC) ratio for high-dose selection in the absence of dose limiting toxicity or other criteria as outlined in ICH S1C(R2) has not been globally accepted as a dose-setting criterion in the rasH2-Tg mouse model. A retrospective evaluation of available data from 53 compounds tested in this model determined that detection of compound-related tumors emerged in all cases within a systemic rodent-to-human exposure ratio up to 50-fold (15). Based on this analysis, it was concluded that a 50-fold plasma exposure ratio (rodent:human) is an adequate

criterion for high-dose selection. Therefore, all criteria for selection of the high dose as specified in ICH S1C(R2) for 2-year rodent carcinogenicity studies are applicable to rasH2-Tg mice, including a plasma exposure ratio, except that the plasma exposure ratio will be 50-fold in rasH2-Tg mice rather than 25-fold as for 2-year studies conducted in standard strains of rodents.

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APPENDIX: CASE STUDIES APPLYING THE WEIGHT OF EVIDENCE APPROACH

Preamble

One outcome of the ICH S1 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 (e.g., viral, microbial) and carcinogenicity data were available with the pharmacologic drug class.
- No identified concerns from secondary pharmacology intended to inform off-target potential for the pharmaceutical.
- Results from chronic toxicity studies indicate no hyperplastic, hypertrophic, atypical cellular alterations, or degenerative/regenerative changes 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.
- The overall assessment of genotoxic potential is concluded to be negative based on criteria from ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012).
- No evidence of immune modulation or immunotoxicity based on target biology and repeat-dose toxicology studies.

Case studies are provided to illustrate the application of the WoE approach. These cases are provided for illustrative purposes only and are not intended to be prescriptive nor to indicate the sufficiency of data to support a WoE assessment. Cases 1 and 2 are examples of pharmaceuticals for which the key WoE factors were integrated to conclude that a 2-year rat study would not add value to the assessment of human carcinogenic risk. 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 would add value to the assessment of human carcinogenic risk. Case 4 describes a pharmaceutical for which a 2-year rat carcinogenicity study was concluded to not contribute value to the assessment of human carcinogenic risk despite there being no data available for other compounds within the pharmacologic class.

Case 1: An inhibitor of viral replication

Summary

Prospective WoE Assessment

- The carcinogenic potential in both rats and humans is unlikely such that a 2-year rat study would not add value to the assessment of human carcinogenicity risk.
- The compound 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 compound-related carcinogenicity findings.

Supportive WoE Factors

Target Biology

- Non-mammalian (viral) target excludes intentional alteration of potential mammalian carcinogenic pathways.
- No compound-related carcinogenicity findings in 2-year rat studies conducted with other compounds with the same viral replication target.

Secondary Pharmacology

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

Histopathology Data from Chronic Studies

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 compound-related histopathologic findings observed in standard battery of tissues.

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-Observed-Adverse-Effect-Level for these effects 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 Effects

- No compound-related findings on endocrine and reproductive organ weights or histopathology.

Genotoxicity

- No evidence of genotoxic potential based on criteria from ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012).

Immune Modulation

- No compound-related changes in clinical pathology or histopathology of immune tissues (e.g., lymph nodes, spleen, thymus, bone marrow).

Additional Investigations

- No data available

Case 2: An antagonist of a neuronal G-protein coupled receptor

Summary

Prospective WoE Assessment

- The carcinogenic potential is unlikely in humans but likely in rats through well-recognized mechanisms shown to be human irrelevant, such that a 2-year rat study would not add value to the assessment of human carcinogenic risk.
- The potential for rodent-specific liver and thyroid tumors was based on the toxicology observed in the chronic rat study and on tumor outcome with the pharmacological class. Hormonal effects due to target pharmacology occurred at high multiples of human exposure and were not considered a human carcinogenic risk. Fluorosis, a potential carcinogenic risk, was observed in rats due to release of fluoride from the compound; however, release of fluoride from the compound was not observed in humans.

2-year Rat Study Results

- The 2-year rat study demonstrated hepatocellular hypertrophy but no compound-related carcinogenicity findings.

Supportive WoE Factors

Target Biology

- Predominate receptor expression in brain with lower expression in some peripheral tissues, similar across species.
- Receptor activation increases adrenocorticotropic hormone (ACTH) release from pituitary secondary to hypothalamic production of adrenocorticotropin-releasing hormone.
- Target knock-out mice showed no findings related to carcinogenicity.
- A 2-year rat study with a comparable compound did not identify a carcinogenic effect that could be ascribed to the intended pharmacological target (see secondary pharmacology section for off-target effects).

Secondary Pharmacology

- Antagonist binding interaction identified for one off-target receptor with Ki 8-fold higher than Cmax at maximum clinical dose. Known pharmacology of off-target receptor not associated with tumorigenesis.
- Thyroid follicular cell adenoma/carcinoma was observed in a 2-year rat study with a comparable compound which was associated with increased thyroid stimulating hormone and ascribed to an off-target pathway related to drug metabolism.

Histopathology Data from Chronic Studies

Rat Study

- Increased liver hypertrophy and organ weight at 50-fold to 74-fold human exposure.
- Increased thyroid follicular hypertrophy at 170-fold to 670-fold human exposure.

Non-rodent Study

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

Hormonal Effects

- Reduced adrenal weight without histopathological correlates and reduced ACTH level at > 74-fold human exposure in the 6-month rat study, consistent with inhibition of drug target.
- 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 suppression of luteinizing hormone and gonadotropin release associated with inhibition of the drug target.
- No treatment-related changes observed in reproductive organ weight or histopathology in 6-month rat study.

Genotoxicity

- No evidence of genotoxic potential of parent or major human metabolite based on criteria from ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012)) .

Immune Modulation

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

Additional Investigations

- Induction of Cytochrome P450 (CYP)1A2 and CYP3A1 demonstrated.
- Bone and teeth fluorosis related to release of fluoride from the compound in rats and demonstrated not to occur in humans.

Case 3: An inhibitor of a ubiquitously expressed serine/threonine kinase (novel target)

Summary

Prospective WoE Assessment

- The carcinogenic potential in humans is uncertain and a 2-year rat carcinogenicity study would add value to the assessment of human carcinogenic risk.
- Carcinogenic uncertainty is related to the complex target pharmacology (e.g., inhibition of cellular apoptosis), the lack of precedent with the drug target, and histopathological changes of concern with inadequate mechanistic explanation from the 6-month rat study which are supported by similar findings in cynomolgus monkeys. While the immune toxicology findings in monkeys (i.e., suppression of T cell-dependent antigen response) contributed to the assessment of human carcinogenicity risk, this finding was not expected to be further informed by a rat carcinogenicity study.

2-year Rat Study Results

- Increased incidence, lethality, and reduced latency of pituitary tumors was observed in both sexes and may be attributed to target pharmacology. The outcome of the 2-year rat study contributed to the overall assessment of human carcinogenic risk.

Supportive WoE Factors

Target Biology

- 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 2-year rodent or 6-month transgenic mouse studies.

Histopathology Data from Chronic Studies

Rat Study

- Increased incidence and severity of renal basophilic tubules, eosinophilic droplets, and brown pigment in renal cortex starting at 14-fold human exposure. Human relevance of lesions was not addressed.
- Chronic irritation of limiting ridge in non-glandular stomach at 39-fold human exposure. Human relevance of lesions was not addressed.
- Increased liver weight without microscopic correlates.

Non-rodent Study

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

Hormonal Effects

- Increased adrenal weight and cortical hypertrophy in rats at 17-fold human exposure. Human relevance of lesions was not addressed.

Genotoxicity

- No evidence of genotoxic potential of parent or major human metabolite based on criteria from ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012).

Immune Modulation

- In monkeys, suppression of T cell-dependent antigen response occurred with no effect on natural killer cell cytotoxicity or granulocyte function.
- Decreased lymphoid cellularity observed in spleen, thymus, lymph nodes at 12-fold human exposure.

Additional investigations

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

Case 4: An inhibitor of a prostaglandin receptor (novel target)

Summary

Prospective WoE Assessment

- The carcinogenic potential in both rats and humans is unlikely such that a 2-year rat study would not add value to the assessment of human carcinogenic risk.
- The drug target is not associated with a role in cancer development, histopathological findings were not observed in the 6-month rat study at a > 50-fold margin of human exposure. Secondary pharmacology also indicated high target selectivity for the compound.

2-year Rat Study Results

- No compound-related carcinogenicity findings.

Supportive WoE Factors

Target Biology

- Receptor activation on innate immune cells is associated with allergic inflammatory responses and available data do not suggest a role in carcinogenesis.
- Knock-out mice lacking the drug target showed no histological abnormalities or effects on immune function during one year of observation.

Secondary pharmacology

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

Histopathology Data from Chronic Studies

Rat Study

- No proliferative changes observed in any organ or tissue at the highest dose tested (~ 54-fold human exposure).

Non-rodent Study

- No proliferative changes in any organ or tissue at the highest dose tested (~ 45-fold human exposure) in repeated-dose toxicity studies of up to 39 weeks.

Hormonal Effects

- No compound-related findings on endocrine and reproductive organ weights or histopathology.

Genotoxicity

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

Immune Modulation

- In the 6-month rat toxicity study, there were no effects on immune function (including in a T cell-dependent antibody response assay) or adverse effects on lymphocyte subsets at the highest dose tested (~ 54-fold human exposure).

Additional Investigations

- No data available.