16 September 2022
EMA/774371/2022
Committee for Medicinal Products for Human Use

ICH guideline S1B(R1) on testing for carcinogenicity of pharmaceuticals

Step5

Transmission to CHMP	25 February 2016
Adoption by CHMP	25 February 2016
Release for public consultation	31 March 2016
End of consultation (deadline for comments)	31 December 2021
Final adoption by CHMP	16 September 2022
Date of coming into effect	16 March 2023

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

S1B(R1) Document History

First Codification	History	Date	New Codification November 2005
S1B	Approval by the Steering Committee under *Step 2* and release for public consultation.	1 May 1996	S1B
S1B	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	16 July 1997	S1B

Current Step 4 version of the S1B(R1)

Code	History	Date
S1B(R1)*	Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation.	10 May 2021
S1B(R1)*	Endorsement by the Regulatory Members of the ICH Assembly under *Step 4* and integration with the S1B Guideline.	4 August 2022

**This addendum is complementary to the S1 Guidelines (S1A, S1B and S1C(R2)) and is not intended to replace the existing S1B Guideline.*

ICH guideline S1B(R1) on testing for carcinogenicity of pharmaceuticals

Table of contents

Part I:
Testing for carcinogenicity of pharmaceuticals

ICH Harmonised Tripartite Guideline

Having reached *Step 4* of the ICH Process at the ICH Steering Committee meeting on

16 July 1997, this guideline is recommended for adoption to the three regulatory parties to ICH

1. Objective

This document provides guidance on approaches for evaluating the carcinogenic potential of pharmaceuticals.

2. Background

Historically, the regulatory requirements for the assessment of the carcinogenic potential of pharmaceuticals in the three regions (E.U., Japan, U.S.) provided for the conduct of long-term carcinogenicity studies in two rodent species, usually the rat and the mouse. Given the cost of these studies and their extensive use of animals, it is in keeping with the mission of ICH to examine whether this practice requiring long term carcinogenicity studies in two species could be reduced without compromising human safety.

This guideline should be read in conjunction with other guidelines (see Annex), especially:

S1.A: Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals.

S1.C: Dose Selection for Carcinogenicity Studies of Pharmaceuticals.

Long-term rodent carcinogenicity studies for assessing the carcinogenic potential of chemicals (including pharmaceuticals) to humans are currently receiving critical examination. Since the early 1970's, many investigations have shown that it is possible to provoke a carcinogenic response in rodents by a diversity of experimental procedures, some of which are now considered to have little or no relevance for human risk assessment. This guideline outlines experimental approaches to the evaluation of carcinogenic potential that may obviate the necessity for the routine conduct of two long-term rodent carcinogenicity studies for those pharmaceuticals that need such evaluation. The relative individual contribution of rat and mouse carcinogenicity studies and whether the use of rats or mice alone would result in a significant loss of information on carcinogenicity relevant to human risk assessment has been addressed by six surveys of the data for human pharmaceuticals. The surveys were those of the International Agency for Research on Cancer (IARC), the U.S. Food and Drug Administration (FDA), the U.S. Physicians' Desk Reference (PDR), the Japanese Pharmaceutical Manufacturers' Association (JPMA), the EU Committee for Proprietary Medicinal Products (CPMP), and the UK Centre for Medicines Research (CMR). The dimensions of these surveys and the principal conclusions of the analyses can be found in the Proceedings of the Third International Conference (1995) on Harmonization.

Positive results in long-term carcinogenicity studies that are not relevant to the therapeutic use of a pharmaceutical present a dilemma to all parties: regulatory reviewers, companies developing drugs and the public at large. The conduct of one long-term carcinogenicity study (rather than two long term studies) would, in part, allow resources to be diverted to other approaches to uncover potential carcinogenicity relevant to humans. A "weight of evidence" approach, that is use of scientific judgment in evaluation of the totality of the data derived from one long-term carcinogenicity study along with

other appropriate experimental investigations, enhances the assessment of carcinogenic risk to humans.

3. Scope of the guideline

The guideline embraces all pharmaceutical agents that need carcinogenicity testing as indicated in Guideline S1A. For biotechnology-derived pharmaceuticals refer to Guideline S6.

4. The guideline

4.1. Preamble

The strategy for testing the carcinogenic potential of a pharmaceutical is developed only after the acquisition of certain key units of information, including the results of genetic toxicology (Guidelines S2A and S2B), intended patient population, clinical dosage regimen (Guideline S1A), pharmacodynamics in animals and in humans (selectivity, dose-response) (Guideline S1C), and repeated-dose toxicology studies. Repeated-dose toxicology studies in any species (including nonrodents) may indicate that the test compound possesses immunosuppressant properties, hormonal activity, or other activity considered to be a risk factor for humans, and this information should be considered in the design of any further studies for the assessment of carcinogenic potential (see also Note 1).

4.2. Experimental approaches to testing for carcinogenic potential

Flexibility and judgment should be exercised in the choice of an approach which should be influenced by the information cited in the above preamble. Given the complexity of the process of carcinogenesis, no single experimental approach can be expected to predict the carcinogenic potential of all pharmaceuticals for humans.

The basic principle:

The basic scheme comprises one long-term rodent carcinogenicity study, plus one other study of the type mentioned in §4.2.2 that supplements the long term carcinogenicity study and provides additional information that is not readily available from the long term assay.

4.2.1. Choice of species for a long-term carcinogenicity study

The species selected should be appropriate, based on considerations that include the following:

1. Pharmacology.
2. Repeated-dose toxicology.
3. Metabolism (see also Guidelines S1C and S3A).
4. Toxicokinetics (see also Guidelines S1C, S3A, and S3B).
5. Route of administration (e.g., less common routes such as dermal and inhalation).

In the absence of clear evidence favoring one species, it is recommended that the rat be selected. This view is based on the factors discussed in §6.

4.2.2. Additional in vivo tests for carcinogenicity

Additional tests may be either 1 or 2 (see Note 2).

1. Short or medium-term in vivo rodent test systems.

   Possibilities should focus on the use of in vivo models providing insight into carcinogenic endpoints. These may include models of initiation-promotion in rodents, or models of carcinogenesis using transgenic or neonatal rodents (Note 3).

2. A long-term carcinogenicity study in a second rodent species is still considered acceptable (see § 4.2.1 for considerations).

4.2.3. Considerations in the choice of short or medium term tests for carcinogenicity.

Emphasis should be placed on selection of a test method that can contribute information valuable to the overall "weight of evidence" for the assessment of carcinogenic potential. The rationale for this choice should be documented and based on information available at the time of method selection about the pharmaceutical such as pharmacodynamics and exposure compared to human or any other information that may be relevant. This rationale should include a scientific discussion of the strengths and weaknesses of the method selected for the pharmaceutical (see Note 4).

5. Mechanistic Studies

Mechanistic studies are often useful for the interpretation of tumor findings in a carcinogenicity study and can provide a perspective on their relevance to human risk assessment. The need for or the design of an investigative study will be dictated by the particular properties of the drug and/or the specific results from the carcinogenicity testing. Dose dependency and the relationship to carcinogenicity study conditions should be evaluated in these investigational studies. Suggestions include:

5.1. Cellular changes

Relevant tissues may be examined for changes at the cellular level using morphological, histochemical, or functional criteria. As appropriate, attention may be directed to such changes as the dose-relationships for apoptosis, cell proliferation, liver foci of cellular alteration, or changes in intercellular communication.

5.2. Cross validation

Depending on the putative mode of tumorigenic action, investigations could involve measurements of:

- plasma hormone levels, e.g. T3/T4, TSH, prolactin
- growth factors
- binding to proteins such as $\alpha 2\mu$-globulin
- tissue enzyme activity, etc.

In some situations, it may be possible to test a hypothesis of, for example, a hormone imbalance with another study in which the imbalance has been, at least in part, compensated.

5.3. Considerations for additional genotoxicity testing

(see Guidelines S2A and S2B)

Additional genotoxicity testing in appropriate models may be invoked for compounds that were negative in the standard test battery but which have shown effects in a carcinogenicity test with no clear evidence for an epigenetic mechanism. Additional testing can include modified conditions for metabolic activation in in vitro tests or can include in vivo tests measuring genotoxic damage in target organs of tumor induction (e.g., DNA damage and repair tests, 32P-postlabeling, mutation induction in transgenes).

5.4. Modified protocols

Modified protocols may be helpful to clarify the mode of tumorigenic action of the test substance. Such protocols might include groups of animals to explore, for example, the consequence of interrupted dosage regimens, or the reversibility of cellular changes after cessation of dosing.

6. General considerations in the choice of an appropriate species for long term carcinogenicity testing

There are several general considerations which, in the absence of other clear indications, suggest that the rat will normally be the species of choice for a long term carcinogenicity study.

6.1. Information from surveys on pharmaceuticals

In the six analyses, attention was given to data on genetic toxicology, tumor incidence, strain of animal, route and dosage regimen, pharmacological or therapeutic activity, development and/or regulatory status, and, if relevant, reason for termination of development. Inevitably, there was considerable overlap of the data, but that is not necessarily an impediment to drawing valid conclusions.

The main overall conclusions from the analysis were:

1. Although very few instances have been identified of mouse tumors being the sole reason for regulatory action concerning a pharmaceutical, data from this species may have contributed to a "weight of evidence" decision and in identifying agents that caused tumors in two rodent species.
2. Of the compounds displaying carcinogenic activity in only one species, the number of "rat-only" compounds was about double the number of "mouse-only" compounds, implying in a simplistic sense that the rat is more "sensitive" than the mouse.
3. As with other surveys accessible in the literature, the data for pharmaceuticals were dominated by the high incidence of rodent liver tumors. The high susceptibility of mouse liver to nongenotoxic chemicals has been the subject of many symposia and workshops. These have concluded that these tumors may not always have relevance to carcinogenic risk in humans and can potentially be misleading.

6.2. Potential to study mechanisms

The carcinogenic activity of nongenotoxic chemicals in rodents is characterized by a high degree of species, strain, and target organ specificity and by the existence of thresholds in the dose-response relationship. Mechanistic studies in recent years have permitted the distinction between effects that are specific to the rodent model and those that are likely to have relevance for humans. Progress has often been associated with increased understanding of species and tissue specificity. For example, receptor-mediated carcinogenesis is being recognized as of growing importance. Most of these advances are being made in the rat, and only rarely in the mouse.

6.3. Metabolic disposition

Neither rats nor mice would seem, on metabolic grounds, to be *a priori* generally more suitable for the conduct of long term carcinogenicity studies. However, much attention is now being given to pharmacokinetic-pharmacodynamic relationships and rapid progress is occurring in knowledge of the P-450 isozymes that mediate the biotransformation of drugs. Most of this research activity is confined to rats and humans. Therefore, in the near future at least, where specific information on the P-450 isozymes involved in biotransformation is critical for the evaluation it appears that mice would be less likely to provide this mechanistic information.

6.4. Practicality

Pertinent to the above two topics is the question of feasibility of investigative studies. Size considerations alone put the mouse at a severe disadvantage when it comes to the taking of serial blood samples, microsurgery/catheterization, and the weighing of organs. Blood sampling often requires the sacrifice of the animals, with the result that many extra animals may be needed when mice are subject to such investigations.

6.5. Testing in more than one species

Most of the currently available short and medium term in vivo models for carcinogenicity testing involve the use of mice. In order to allow testing in more than one species for carcinogenic potential, when this is considered important and appropriate, the rat will often be used in the long term carcinogenicity study.

6.6. Exceptions

Despite the above considerations, there may be circumstances under which the mouse or another rodent species could be justified on mechanistic, metabolic, or other grounds as being a more appropriate species for the long term carcinogenicity study for human risk assessment (c.f. §4.2.1). Under such circumstances it may still be acceptable to use the mouse as the short term or medium term model.

7. Evaluation of carcinogenic potential

Evidence of tumorigenic effects of the drug in rodent models should be evaluated in light of the tumor incidence and latency, the pharmacokinetics of the drug in the rodent models as compared to humans, and data from any ancillary or mechanistic studies that are informative with respect to the relevance of the observed effects to humans.

The results from any tests cited above should be considered as part of the overall "weight of evidence" taking into account the scientific status of the test systems.

Notes

Note 1. Data from in vitro assays, such as a cell transformation assay, can be useful at the compound selection stage.

Note 2. If the findings of a short or long-term carcinogenicity study and of genotoxicity tests and other data indicate that a pharmaceutical clearly poses a carcinogenic hazard to humans, a second carcinogenicity study would not usually be useful.

Note 3. Several experimental methods are under investigation to assess their utility in carcinogenicity assessment. Generally, the methods should be based on mechanisms of carcinogenisis that are believed relevant to humans and applicable to human risk assessment. Such studies should supplement the long term carcinogenicity study and provide additional information that is not readily available from the long term assay. There should also be consideration given animal numbers, welfare and the overall economy of the carcinogenic evaluation process. The following is a representative list of some approaches that may meet these criteria and is likely to be revised in the light of further information.

1. The initiation-promotion model in rodent. One initiation-promotion model for the detection of hepatocarcinogens (and modifiers of hepatocarcinogenicity) employs an initiator, followed by several weeks of exposure to the test substance. Another multi-organ carcinogenesis model employs up to five initiators followed by several months of exposure to the test substance.
2. Several transgenic mouse assays including the p53+/- deficient model, the Tg.AC model, the TgHras2 model, the XPA deficient model, etc.
3. The neonatal rodent tumorigenicity model.

Note 4. While there may be a number of approaches that will in general meet the criteria described in Note 3 for use as the additional in vivo study, not all may be equally suitable for a particular pharmaceutical. The following are examples of factors that should be considered and addressed in the rationale:

1. Can results from the model provide new information not expected to be available from the long-term study that is informative with respect to hazard identification and/or risk assessment?

2. Can results from the model address concerns related to the carcinogenic process arising from prior knowledge of the pharmaceutical or compounds with similar structures and/or mechanisms of action? These concerns may include genotoxic, mitogenic, promotional, or receptor-mediated effects, etc.

3. Does the metabolism of the pharmaceutical shown in the animal model affect the evaluation of carcinogenic risk for humans?

4. Is adequate systemic or local exposure attained in relation to human exposure?

5. How extensively has the model been evaluated for its intended use? Prior to using any new in vivo methods in testing the carcinogenic potential of pharmaceuticals for humans, it is critical that the method be evaluated for its ability to contribute to the weight of evidence assessment. Many experimental studies are in progress (1997) to evaluate the new short or medium tests for carcinogenic potential. These include selected pharmaceuticals with known potencies and known mechanism of carcinogenic activity in rodents, and also putative human non-carcinogens. When the results of these studies become available, it may be possible to offer clearer guidance on which of these tests have the most relevance for cancer assessment in humans.

ANNEX: Other ICH Guidelines Cited

Guideline S2A: Notes for Guidance on Specific Aspects of Regulatory Genotoxicity Tests.

Guideline S2B: A Standard Battery of Genotoxicity Testing of Pharmaceuticals.

Guideline S3A: Notes for Guidance on Toxicokinetics. The Assessment of Systemic Exposure in Toxicity Studies.

Guideline S3B: Guidance on Repeat-Dose Tissue Distribution Studies.

Guideline S6: Preclinical Testing of Biotechnology-derived Pharmaceuticals.

Part II:
Addendum to testing for carcinogenicity for pharmaceuticals

Preamble

This Addendum is to be used in close conjunction with *ICH S1A Guideline on the Need for Carcinogenicity Studies of 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 applies to all pharmaceuticals that need carcinogenicity testing as described in Guideline S1A. For biotechnology-derived pharmaceuticals, refer to Guideline S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

1.2. Purpose of the Addendum

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 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 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[1] while all other aspects of the recommendations for high dose selection in S1C(R2) Guideline 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.

1.3. Background

While the S1B Guideline 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 the ICH S1B Guideline, 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

[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 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 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 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.

carcinogenic potential could have 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), a subsequent international prospective study was conducted under ICH *S1(R1) Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals – Regulatory Notice Document*. The process and several status updates reporting results are posted and available at the ICH website (10-14). Carcinogenicity assessment documents (CADs) and associated data from 2-year rat carcinogenicity studies for 45 compounds were received and evaluated by regulatory members of the ICH EWG. 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 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 (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.

2. A weight of evidence approach to assess the human carcinogenic potential of 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 carcinogenic potential of the pharmaceutical in humans is:

- likely, such that a 2-year rat carcinogenicity study would not add value; or
- unlikely, such that a 2-year rat carcinogenicity study would not add value[3] ; or
- 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).

[2] Methods and results of the ICH S1 prospective evaluation study will be summarized in a future publication.

[3] 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.

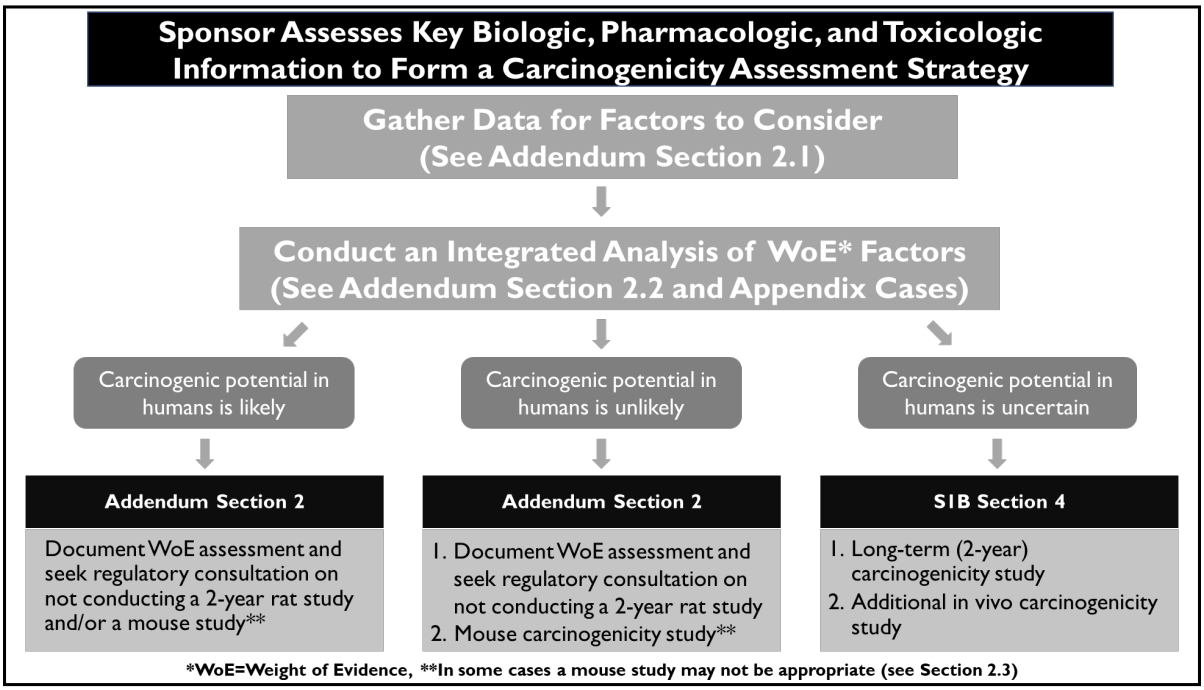

Figure 1. Flow scheme outlining key steps and options in developing a carcinogenicity assessment strategy and determining the added value of a 2-year rat study. Note that key biologic, pharmacologic, and toxicologic information should be assessed even when taking the ICH S1B approach that utilizes a 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.

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 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),
3. histopathology data[4] from repeated-dose toxicity 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,

[4] 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 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

4. evidence for hormonal perturbation[5], 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,

5. genetic toxicology study data using criteria from ICH S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use; equivocal genotoxicity data that cannot be resolved in accordance with ICH S2(R1) recommendations increases uncertainty with respect to the carcinogenic potential,

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

The above WoE factors may be sufficient to conclude whether or not 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), 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).

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 included in the WoE document.

2.2. Integration of WoE Factors for Assessing Human Carcinogenic Risk

An integrated analysis of the WoE factors described above should be used to determine whether or not 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).

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.

[5] 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.

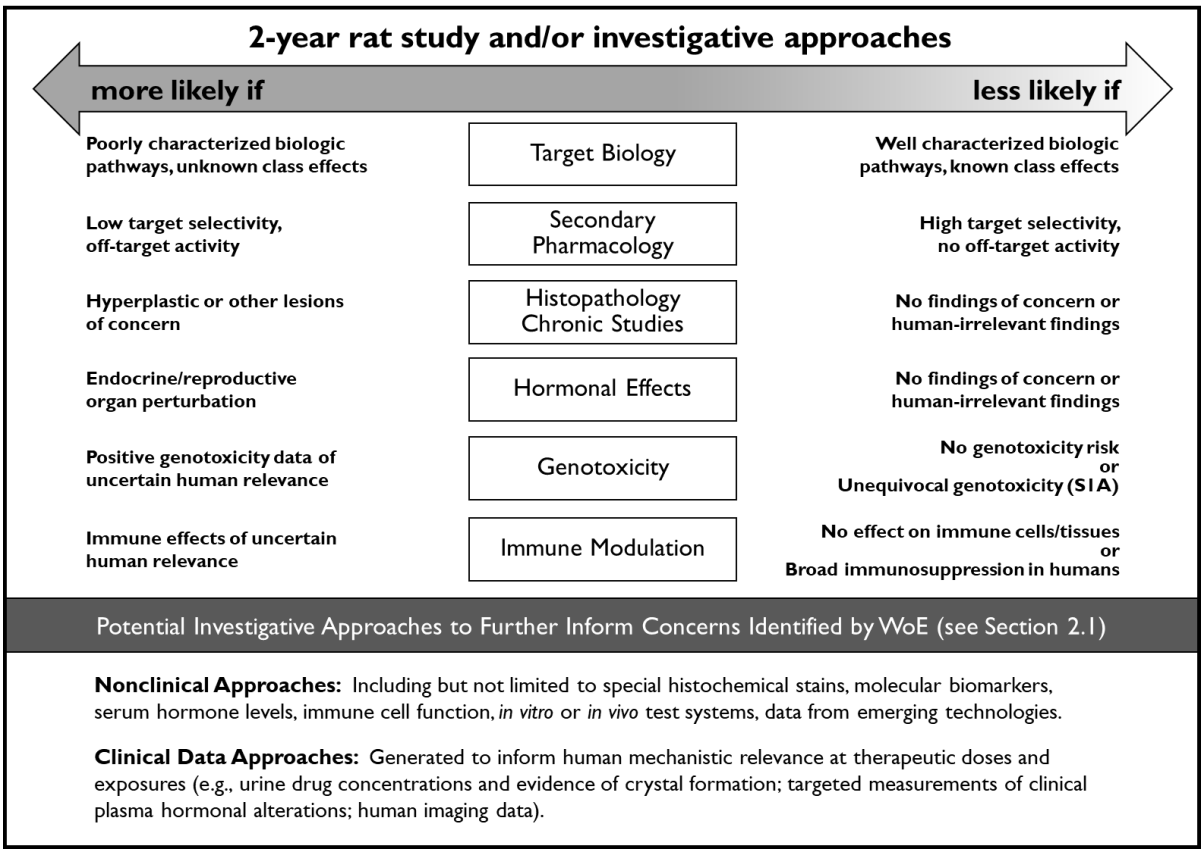

Figure 2. Integration of key WoE factors and potential investigative approaches to further inform on the value of conducting a 2-year rat study for assessment of human carcinogenic risk. When all WoE attributes align towards the right side of the figure, a conclusion that a 2-year rat study would not 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 (*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 in regard to 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.

2.3. Mouse Carcinogenicity Studies

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.

3. Clarification on criteria for high dose selection based on exposure for rash2-tg mouse carcinogenicity studies

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 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.

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. Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals –Regulatory Notice Document. ICH, 2016. URL: https://database.ich.org/sites/default/files/S1%28R1%29_EWG_RND.pdf (last accessed 31 May 2022)

11. The ICHS1 Regulatory Testing Paradigm of Carcinogenicity in Rats - Status Report Introduction Background: The RND Hypothesis and the Prospective Evaluation Study. ICH, 2016. URL: https://database.ich.org/sites/default/files/S1%28R1%29%20EWG_StatusReport_Mar2016.pdf. (last accessed 31 May 2022)

12. The ICHS1 Regulatory Testing Paradigm of Carcinogenicity in Rats: Status Report December 2017. ICH, 2017. URL: https://database.ich.org/sites/default/files/S1%28R1%29%20EWG_StatusReport_Dec2017.pdf. (last accessed 31 May 2022)

13. The ICHS1 Regulatory Testing Paradigm of Carcinogenicity in Rats: Status Report 2019. ICH, 2019. URL: https://database.ich.org/sites/default/files/S1_StatusReport_2019_0802.pdf. (last accessed 31 May 2022)

14. The ICHS1 Regulatory Testing Paradigm of Carcinogenicity in Rats: Status Report 2021. ICH, 2021. URL: https://database.ich.org/sites/default/files/S1_StatusReport_2021_0823.pdf. (last accessed 31 May 2022)

15. Hisada S, Tsubota K, Inoue K, Yamada H, Ikeda T, Sistare FD. Survey of tumorigenic sensitivity in 6-month rasH2-Tg mice studies compared with 2-year rodent assays. J Toxicol Pathol 2022;35:53–73.

16. 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.

17. 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: 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 S2(R1) Guidance.
- 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 S2(R1) Guidance.

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 S2(R1) Guidance.

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 CYP1A2 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 S2(R1) Guidance.

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) Guidance.

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