

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

**ICH HARMONISED GUIDELINE**

**ADDENDUM TO THE GUIDELINE ON TESTING  
FOR CARCINOGENICITY OF  
PHARMACEUTICALS  
S1B(R1)**

Draft version

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*\*This addendum is complementary to the S1 Guidelines (S1A, S1B and S1C(R2)) and is not intended to replace the existing S1B Guideline. At Step 4 of the ICH process, this addendum will be integrated with the S1B Guideline.*

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**ICH HARMONISED GUIDELINE**  
**ADDENDUM TO THE GUIDELINE ON TESTING FOR**  
**CARCINOGENICITY OF PHARMACEUTICALS**

**ICH S1B(R1)**

**ICH Consensus Guideline**

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1 **PREAMBLE**

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

6 **1. INTRODUCTION**

7 **1.1 Scope of the Addendum**

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

10 **1.2 Purpose of the Addendum**

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

18 Application of this integrative approach would reduce the use of animals in accordance with the  
19 3Rs (reduce/refine/replace) principles, and shift resources to focus onto generating more  
20 scientific mechanism-based carcinogenicity assessments, while promoting safe and ethical  
21 development of new small molecule pharmaceuticals.

22 **1.3 Background**

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

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<sup>1</sup> 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.

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

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

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

## 46 **2. A WEIGHT OF EVIDENCE APPROACH TO ASSESS THE HUMAN** 47 **CARCINOGENIC POTENTIAL OF SMALL MOLECULE PHARMACEUTICALS**

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

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

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<sup>2</sup> 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.

<sup>3</sup> 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).

- 56 well recognized mechanisms known to be human irrelevant); or  
57 • uncertain with respect to the carcinogenic potential for humans, and a 2-year rat  
58 carcinogenicity study is likely to add value to human risk assessment.  
59

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

## 64 **2.1 Factors to consider for a WoE assessment**

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

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

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<sup>4</sup> 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.

<sup>5</sup> 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.

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

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

## 100 2.2 Integration of WoE Factors for Assessing Human Carcinogenic Risk

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

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

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

## 122 2.3 Mouse Carcinogenicity Studies

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

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<sup>6</sup> 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.

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

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

132 In practice, a plasma exposure (AUC) ratio for high dose selection in the absence of dose  
 133 limiting toxicity or appropriate use of other dose setting criteria as outlined in ICH S1C(R2) in  
 134 this model, has not been globally accepted as an endpoint. Therefore, available data from  
 135 experience with 50 compounds evaluated in the rasH2-Tg mouse model were analyzed and the  
 136 conclusion reached that there was no value in exceeding a 50-fold plasma AUC exposure ratio  
 137 (rodent:human) to support carcinogenicity assessment. Therefore, all criteria for selection of  
 138 the high dose for carcinogenicity studies as specified in S1C(R2) for 2-year rodent studies are  
 139 applicable to rasH2-Tg, including an AUC plasma exposure ratio, except that the exposure  
 140 ratio will be 50-fold in rasH2-Tg rather than 25-fold as for 2-year studies conducted in wild  
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## ICH S1B(R1) Guideline

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182 **APPENDIX 1: CASE STUDIES APPLYING THE WEIGHT OF EVIDENCE**  
 183 **APPROACH**

184  
 185 **Preamble**  
 186

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

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

206  
 207 Although rasH2-Tg mouse study results were recommended when available as a WoE element  
 208 in the initial RND, they did not significantly contribute to the prediction of the 2-year rat  
 209 carcinogenicity study outcome. Therefore, a rasH2-Tg mouse study is not expected to be  
 210 completed to support a WoE assessment. However, if rasH2-Tg mouse study results are  
 211 available, they should be discussed in the assessment.

212  
 213 A series of case studies are provided to illustrate the application of the WoE approach. These  
 214 cases are provided for illustrative purposes only and are not intended as guidance to indicate  
 215 the sufficiency of data to support a WoE assessment. Cases 1 and 2 describe the key WoE  
 216 factors for that pharmaceutical and how the data were integrated to conclude that a 2-year rat  
 217 study would not add value to the assessment of carcinogenic risk. In contrast to these cases,  
 218 Case 3 describes how data from the WoE factors were integrated to conclude that the  
 219 carcinogenic potential for humans was uncertain, and a 2-year rat carcinogenicity study was  
 220 likely to add value to human risk assessment. Case 4 describes a molecule for which a 2-year  
 221 rat carcinogenicity study was concluded to not contribute value to human carcinogenicity  
 222 assessment despite there being no data available for other molecules within the pharmacologic  
 223 class.

224  
 225 **Case 1: A small molecule inhibitor against a non-mammalian target**  
 226

227 **Prospective WoE Assessment: Concluded by all DRAs and Sponsor as likely not to be carcinogenic**  
 228 **in both rats or humans such that a 2-year rat study would not add value**  
 229

230 **Rationale**

231 The WoE analysis supports the conclusion that the molecule was sufficiently studied at high

232 exposure margins, and cause-for-concern was not identified for any of the WoE factors.

233

234 **2-year Rat Study Results: No test article related neoplastic findings were present in the 2-year rat**  
235 **study.**

236

237 **WoE Criteria**

238

239 Knowledge of intended drug target and pathway pharmacology relative to carcinogenesis

240 • Non-mammalian target excludes intentional alteration of potential mammalian  
241 carcinogenic pathways.

242 • No evidence of carcinogenic outcome in 2-year rat studies conducted with other  
243 compounds with the same non-mammalian pharmacological target

244

245 Secondary Pharmacology Screen

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

248

249 General Toxicology from Chronic Rat Study

250 • Chronic (6-month) toxicology study in Wistar rats dosed to saturation of absorption,  
251 achieving up to a 31-fold margin to human exposure.

252 • No evidence of human specific major metabolites.

253 • No treatment-related histopathologic findings observed in standard battery of tissues

254

255 General Toxicology from Chronic Non-rodent Study

256 • Chronic administration (9-month) to non-human primates identified bile duct  
257 hyperplasia and hepatocellular hypertrophy, with reactive neutrophils and  
258 regenerative hyperplasia. A No-Adverse-Effect-Level was identified which provided  
259 a 5-fold margin to human exposure.

260 • Further evaluation in rats would not provide useful information, as similar findings  
261 were not observed in the chronic rat study.

262

263 Hormonal Perturbation

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

265

266 Genetic Toxicology

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

268

269 Immune Toxicology

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

272

273 Additional Special Investigations

274 • No data available

275

276

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

278

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

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**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.

332 Genetic Toxicology

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

335  
336 Immune Toxicology

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

340  
341 Additional Special Investigations

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

345  
346  
347 **Case 3: A first-in-class small molecule inhibitor of a ubiquitously expressed**  
348 **serine/threonine kinase**

349  
350 **Prospective WoE Assessment: Unanimously concluded to be uncertain with respect to the**  
351 **carcinogenic potential for humans, and a 2-year rat carcinogenicity study is likely to add value to**  
352 **human carcinogenicity assessment**

353  
354 **Rationale**

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

361  
362 **2-year Rat Study Results: The 2-year rat study demonstrated an increased incidence, lethality,**  
363 **and reduced latency of pituitary tumors in both sexes. This carcinogenic outcome in rats would**  
364 **contribute to the overall assessment of human carcinogenic potential.**

365  
366 **WoE Criteria**

- 367 Knowledge of intended drug target and pathway pharmacology relative to carcinogenesis
- 368 • Target activation by inflammation-related oxidative stress promotes cellular apoptosis  
369 and is linked to control of cell proliferation; target inhibition suppresses apoptotic  
370 signaling and impacts cell proliferation, theoretically promoting cancer growth.
  - 371 • Drug target displays tissue-dependent roles in cancer development, both promotion  
372 and suppression, in animal models.
  - 373 • No data available on tumor outcome from target inhibition in long term rodent or  
374 short term transgenic mouse studies

375  
376  
377 General Toxicology from Chronic Rat Study

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

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

386

387 General Toxicology from Chronic Non-rodent Study

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

392

393 Hormonal Perturbation

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

396

397 Immune Toxicology

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

401

402 Genetic Toxicology

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

405

406 Additional Special investigations

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

408

409

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

411

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

414

415 **Rationale**

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

421

422 **2-year Rat Study Results: The 2-year rat carcinogenicity study did not demonstrate a dose-related**  
423 **increase in tumors.**

424

425 **WoE Criteria**

426

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

## ICH S1B(R1) Guideline

- 429
- 430
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- 436
- 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.

437

### 438 Secondary pharmacology screen

- 439
- 440
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- 443
- 444
- 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.

### 445 General Toxicology from Chronic Rat Study

- 446
- 447
- 448
- 449
- 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.

450

### 451 General Toxicology from Chronic Non-rodent Study

- 452
- 453
- 454
- 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).

455

### 456 Hormonal Perturbation

- 457
- No treatment-related findings on reproductive organ weights or histopathology.

458

### 459 Genetic Toxicology

- 460
- No evidence of genotoxic potential based on criteria from ICH S2(R1) Guidance.

461

### 462 Immune Toxicology

- 463
- 464
- 465
- 466
- 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).

467

### 468 Additional Special Investigations

- 469
- Not performed.