Risk Evaluation of Nongenotoxic Carcinogenic Pharmaceuticals:

Impact of the ICH S1B Addendum: a European Viewpoint

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Carcinogenicity of human pharmaceuticals

Rethinking the Rodent Carcinogenicity testing?

International Council on Harmonisation of Technical Requirements on Pharmaceuticals for human use

(ICH) S1 Regulatory Notice Document

Athens, May 2022
50% of all pharmaceuticals used for long-term therapies induce rodent cancer. Van Oosterhout et al (1997); Contreras, Jacobs, DeGeorge (1997)

Data from Van Oosterhout:
- Genotoxic: 6/88 (all positives out of 171)
- Estrogenic (hormonal): 4
- Immunosuppression: 2

These might be the only mechanisms with relevance to humans? Why conducting so many studies?
These studies from European and US regulators are conducted to support the writing of ICH S1B: There is room for replacing the long-term mouse for other approaches:

**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 carcinogenicity study and provides additional information that is not readily available from the long term assay.

4.2.2. Additional in vivo tests for carcinogenicity
   a: Short or medium-term in vivo rodent test systems. .... carcinogenic endpoints. These may include models of initiation-promotion in rodents or models of carcinogenesis using transgenic or neonatal rodents
   b: A long-term carcinogenicity study in a second rodent species is still considered acceptable.
## Carcinogenicity of human Pharmaceuticals

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mice</td>
<td>rats</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2011: 15 years after writing S1B

- Data from EU show that nothing has changed. The pattern of rodent carcinogenesis in long-term therapies on the market is similar.

- Transgenic mice (p53+/- and TgRasH2) are being accepted, but the impact is low. It’s use is infrequent.

- Continuous discussion to reduce the long-term rat study.
  - Study from Reddy et al, 2010 with 60 compounds from Merck Research Laboratories
  - Study from Sistare et al, 2011 with > 190 compounds from 13 PhRMA partners
Carcinogenicity of human pharmaceuticals

Rat chronic toxicology studies are good predictors of negative outcome in 2 yr rat carcinogenicity studies:

a) Results derived from 182 compounds across 242 rat chronic tox studies and 182 2-yr rat carcinogenicity studies conducted by 13 pharma companies over 25+ years. In addition: Results derived from 76 IARC 1 & 2a carcinogenic chemicals, and 8 pharmaceuticals withdrawn from the market. - 266 total chemicals -

b) Predictivity on an organ-by-organ basis is poor, but overall negative predictivity is very good on a whole animal basis. NO chronic tox preneoplasia + NO genetox + NO hormonal perturbation signals = NO value added from 2 yr rat carco study.

c) The results of these analyses hold promise in driving to support modifications to current carcinogenicity testing guidelines, while maintaining patient safety, accelerating patient access, and significantly reducing animal testing.

Sistare et al, 2011
The hypothesis...

That a rat chronic tox study will identify an effect that would define the need for completing a 2-yr assay, i.e.,

(+): His in any tissue, genetox positive, or clear evidence of hormonal perturbation ➔ run a 2-year bioassay

(-): His in all tissues, no genetox, and no evidence of hormonal perturbation ➔ conclude no carcinogenic concern & no need to perform the 2-year rat study

Rationale:

1) tumorigenic processes are often dependent on multi-organ participation (e.g., liver/thyroid; pituitary/mammary)

2) tumorigenic cmpds of higher concern are multi-site/-species/-sex; so sensitivity enhanced using “any organ” signal
NEG CARC Proposal PhRMA

- 21-24 False Negatives

- What is the impact of these False Negatives (FN’s)?

- EU position:
  Integrated approach of all cases. Include Pharmacology.

- FN’s are exceptions on the rule
  - The rule is not a real concern or a well-known risk

- Therefore: FN’s does not change eventually the outcome
Carcinogenicity of human pharmaceuticals

Additional evaluation on drug-target

Critical Reviews in Toxicology, 2016

Table 1. Pharmacotherapeutic categories.

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>PhRMA</th>
<th>FDA</th>
<th>JPMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>73</td>
<td>45</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>48</td>
<td>32</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Respiratory</td>
<td>23</td>
<td>18</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Metabolic</td>
<td>28</td>
<td>18</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Hormonal</td>
<td>20</td>
<td>13</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Anti-inflammatory and immunomodulatory</td>
<td>16</td>
<td>15</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Antiviral and antimicrobials</td>
<td>43</td>
<td>35</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Remaining</td>
<td>47</td>
<td>15</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>298</td>
<td>191</td>
<td>44</td>
<td>63</td>
</tr>
</tbody>
</table>
### Table 9. Classes with high percentage of rat carcinogens.

<table>
<thead>
<tr>
<th>Class</th>
<th>Total number of compounds</th>
<th>Compounds with tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat carcinogenicity related to pharmacology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DA₂-antagonists</td>
<td>13</td>
<td>9 (71)</td>
</tr>
<tr>
<td>28 Adrenergic β₂-agonists</td>
<td>5</td>
<td>4 (80)</td>
</tr>
<tr>
<td>31 PPAR-γ agonists</td>
<td>8</td>
<td>7 (88)</td>
</tr>
<tr>
<td>32 HMG-CoReductase inhibitors</td>
<td>5</td>
<td>4 (80)</td>
</tr>
<tr>
<td>35 Estrogen Modulators</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>41 Proton pump inhibitors</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>42 H₂ antagonists</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>44 Vit D analogs</td>
<td>4</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Rat carcinogenicity not related to pharmacology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 5HT₂-antagonists</td>
<td>3</td>
<td>2 (67)</td>
</tr>
<tr>
<td>37 Aromatase inhibitors</td>
<td>5</td>
<td>4 (80)</td>
</tr>
</tbody>
</table>

### Table 10. “Negative” classes.

<table>
<thead>
<tr>
<th>Class</th>
<th>Number</th>
<th>With tumors</th>
<th>No tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 5HT₁b/₄-agonists (triptanes)</td>
<td>4</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>5 5HT₂-agonists</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>6 SSRIs</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>8 NMDA-agonists</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>9 DA/NE-reuptake inhibitors</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>11 GABA-A-agonists</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>14 Ach-esterase-inhibitors</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>15 Ca-agonants</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>16 ACE inhibitors</td>
<td>7</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>17 All antagonists</td>
<td>6</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>18 Phosphodiesterase 5 inhibitors</td>
<td>3</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>19 Adrenergic α₂-agonists</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>21 Beta blockers</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>22 Vasopressin antagonists</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>34 Aldose-reductase inhibitors</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>39 Anti-inflammatory compounds</td>
<td>12</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>43 Anticholinergics</td>
<td>4</td>
<td>–</td>
<td>4</td>
</tr>
</tbody>
</table>
Mixed Outcome classes: How to handle novel targets?

Table 4 Inconclusive classes: **Green probably negative; Red probably positive**

<table>
<thead>
<tr>
<th>Class</th>
<th>Nr.</th>
<th>With Tumors</th>
<th>No Tumors</th>
<th>True positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>- α2δ-agonists</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>+ DA2-agonists</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Musc. M1 agonists</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>- μ-opioid agonists</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>α2a-antagonists</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adrenergic α1 antagonists</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Leukotriene Receptor antagonists</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>- H1 antihistamines</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gliptins</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Phosphodiesterase4 inhibitors</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Testosterone inhibitors</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GnRH-antagonists</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>+ Immunosuppressants/modulators</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>+ Biphosphonates</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
New Hypothesis

When outcome is predictable, studies are not needed

- **Positive prediction**: On the basis of pharmacology. Positive classes support positive prediction. Furthermore, the presence of proliferative signals in histopathology, e.g. hypertrophy, hyperplasia.

- **Negative prediction**: Based on absence of histopathology and negative pharmacological class. This can support an unexpected positive result.
Risk categories assigned to Carcinogenicity Assessment Documents

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

Category 2: the available sets of pharmacologic and toxicologic data indicate that tumorigenic potential for humans is uncertain and rodent carcinogenicity studies are likely to add value to human risk assessment.

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

Category 3b: highly likely not to be tumorigenic in both rats or humans, such that a 2-year rat study would not add value.
Drug Regulatory Agency (DRA) participation in Prospective Evaluation Period

European Medicines Agency (EMA)\(^1\)

Pharmaceuticals and Medical Devices Agency (PMDA)\(^1\)

U.S. Food and Drug Administration (FDA)\(^1\)

Health Canada (HC)\(^2\)

Swiss Agency for Therapeutic Products (SMC)\(^2\)

\(^1\)EMA, PMDA, FDA contributed to Categorization of all submitted CADs.

\(^2\)HC, SMC contributed to Categorization of 32 and 23 submitted CADs, respectively, after entry in PEP.

All DRAs participated in evaluative comparison of CADs to associated rat carcinogenicity study outcomes.
Potential outcome

- Try-out with products in real world
- All sponsors submit a Carcinogenicity Assessment Document (CAD) with a prediction.
- Regulators decide about added value of 2-year rat study.
- At outcome stage of the study the hypothesis of the prediction will be checked, and the added value has been evaluated.
- If verified the guidelines will change.

May 2022: 48 CAD’s
45 Study reports and CAD’s have been discussed
Concordance among DRAs on Sponsor-proposed Category designations for completed CAD/FSR cases

<table>
<thead>
<tr>
<th>CAD Category</th>
<th>Number of CADs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sponsor</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>3a</td>
<td>14</td>
</tr>
<tr>
<td>3b</td>
<td>17</td>
</tr>
</tbody>
</table>
Tumor outcome of 2 year rat studies for cases designated as categories 1, 2, 3A, and 3B by drug regulatory agencies or by sponsors.2-year

<table>
<thead>
<tr>
<th>CAD Category</th>
<th>Sponsor-determined 2-year rat outcome</th>
<th>DRA-determined 2-year rat outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>3a</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>3b</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>
The ICH S1 Regulatory Testing Paradigm of Carcinogenicity in rats.

4th Status Report - August 2021

Conclusion:
The DRAs/EWG concluded that results from the Prospective Evaluation Study can support pursuing an S1B addendum that describes a WoE-based assessment of carcinogenic risk for small molecule pharmaceuticals that have attributes similar to those observed in the unanimous Category 3 cases; however, for a significant number of programs, the 2-year rodent bioassay continued to provide value and remained the appropriate path
Sponsor Assesses Key Biologic, Pharmacologic, and Toxicologic Information to Form a Carcinogenicity Assessment Strategy

Gather Data for Factors to Consider (See Addendum Section 2.1)

Conduct an Integrated Analysis of WoE* Factors (See Addendum Section 2.2 and Appendix Cases)

Carcinogenic potential in humans is likely

Carcinogenic potential in humans is unlikely

Carcinogenic potential in humans is uncertain

Addendum Section 2
Document WoE assessment and seek regulatory consultation on not conducting a 2-year rat study and/or a mouse study

Addendum Section 2
1. Document WoE assessment and seek regulatory consultation on not conducting a 2-year rat study
2. Mouse carcinogenicity study

SIB Section 4
1. Long-term (2-year) carcinogenicity study
2. Additional in vivo carcinogenicity study

*WoE=Weight of Evidence, ¹ In some cases a mouse study may not be appropriate (see Section 2.3)
2-year rat study and/or investigative approaches

**more likely if**

- Poorly characterized biologic Pathways, unknown class effects
- Low target selectivity, off-target activity
- Hyperplastic or other lesions of concern
- Endocrine/reproductive organ perturbation
- Positive genotoxicity data of uncertain human relevance
- Immune effects of uncertain human relevance

**less likely if**

- Well characterized biologic pathways, known class effects
- High target selectivity, no off-target activity
- No findings of concern or human-irrelevant findings
- No findings of concern or human-irrelevant findings
- Unequivocal genotoxicity (S1A)
- "No effect on immune cells/tissues
- "Broad immunosuppression in humans

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**Potential Investigative Approaches to Further Inform Concerns Identified by WoE (see Section 2.1)**

**Nonclinical Approaches:** Including but not limited to special histochemical stains, molecular biomarkers, serum hormone levels, immune cell function, in vitro or in vivo test systems, data from emerging technologies.

**Clinical Data Approaches:** 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).
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

Histopathology Data from Chronic Studies
Rat Study
- Chronic (6-month) toxicology study 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.
- Similar findings were not observed in the chronic rat study. → No added value to conduct another rat study.

Hormone perturbation, Genetic toxicology, Immune Toxicology: No changes to be reported.
Summary
Prospective WoE Assessment (Sponsor 3B/DRA 3B, Unanimously)
• 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.
**Target Biology**
- Predominate receptor expression in brain with lower expression in some peripheral tissues, similar across species
- Receptor activation increases adrenocorticotropin 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 target 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

**Immune Modulation**

- No treatment-related changes

**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.
Summary
Prospective WoE Assessment (Sponsor 3A/DRA 3A, Unanimously)

- 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.
Case 3 An inhibitor of an ubiquitously expressed serine/threonine kinase (novel target)

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.

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.

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

Additional investigations
• Increases in hepatic enzymes CYPs 1A, 3A, and 2B demonstrated.
Case 3 An inhibitor of an ubiquitously expressed serine/threonine kinase (novel target)

Summary
Prospective WoE Assessment (Sponsor 2/DRA 2, Unanimously)

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

**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 of 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 up to 39-weeks.
Case 4: An inhibitor of a prostaglandin receptor (novel target)

**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 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.
- study at a > 50-fold margin of human exposure. Secondary pharmacology also indicated high target selectivity for the compound.
Case 4: An inhibitor of a prostaglandin receptor (novel target)

### Summary
Prospective WoE Assessment (Sponsor 3B/DRA 3B, Unanimously, despite novel target)

- 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.
**Original Article**

**Survey of tumorigenic sensitivity in 6-month rasH2-Tg mice studies compared with 2-year rodent assays**

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**Abstract:** The pharmacokinetic endpoint of a 25-fold increase in human exposure is one of the specified criteria for high-dose selec-tion for 2-year carcinogenicity studies in rodents according to ICH S1C(R2). However, this criterion is not universally accepted for 6-month carcinogenicity tests in rasH2-Tg mice. To evaluate an appropriate multiple for rasH2-Tg mice, we evaluated data for 53 compounds across three categories of rasH2-Tg mouse-positive [(1) genotoxic and (2) non-genotoxic] carcinogens and rasH2-Tg mouse-negative [(3) non-genotoxic carcinogens with clear or uncertain human relevance; (4) genotoxic rodent-specific carcinogens; and (5) non-carcinogens], and surveyed their tumorigenic activities and high doses in rasH2-Tg mice and 2-year rodent models. Our survey indicated that area under the curve (AUC) margins (AMs) or body surface area-adjusted dose ratios (DRs) of tumorigenesis in rasH2-Tg mice to the maximum recommended human dose (MRHD) were 0.05- to 5.2-fold in 6 category (1) compounds with small differences between models and 0.2- to 47-fold in 7 category (2) including three 2-year rat study-negative compounds. Among all 53 compounds, including 40 compounds of the rasH2-Tg mouse-negative category (3), (4), and (5), no histopathologic risk factors for rodent neoplasia were induced only at doses above 50-fold AM or DR in rasH2-Tg mice except for two compounds, which induced hyperplasia and had no relationship with the tumors observed in the rasH2-Tg mouse or 2-year rodent studies. From the results of these surveys, we con-firmed that exceeding a high dose level of 50-fold AM in rasH2-Tg mouse carcinogenicity studies does not appear to be of value. (DOI: 10.1293/tox.2021-0031; J Toxicol Pathol 2022; 35: 53–73)

**Key words:** rasH2-Tg mouse, carcinogenicity, ICH S1C guideline, high dose selection, pharmacokinetic parameters, maximum recommended human dose
Impact Role of EU Directive 2010/63 on the protection of animals used for scientific purposes

Directive 2010/63/EU unambiguously fosters the application of the principle of the 3Rs (replacement, reduction and refinement) by stating in article 4 that:

1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure.

2. Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project.

European Parliament
2019-2024

TEXTS ADOPTED

P9_TA(2021)0387
Plans and actions to accelerate a transition to innovation without the use of animals in research, regulatory testing and education
European Parliament resolution of 16 September 2021 on plans and actions to accelerate the transition to innovation without the use of animals in research, regulatory testing and education (2021/2784(RSP))
Role of genomics

HESI eSTAR Committee

A Collaborative Initiative to Establish Genomic Biomarkers for Assessing Tumorigenic Potential to Reduce Reliance on Conventional Rodent Carcinogenicity Studies


Figure 2. Strategy to reduce the reliance on the 2-year rodent bioassay to identify carcinogens. The strategy is put into the context of the molecular initiating events (MIE) and key events (KE) causal for induction of liver tumors in rats as an example. The overall strategy could be applied to other tissues with sufficient information about AOPs important in tumor induction. The figure outlines the MIEs (left side) and certain KEs (middle) that could be measured using genomic interrogation techniques including gene expression biomarkers and error-corrected sequencing.
Physiologically Relevant Estrogen Receptor Alpha Pathway Reporters for Single-Cell Imaging-Based Carcinogenic Hazard Assessment of Estrogenic Compounds

Britt Duijndam,† Annabel Goudriaan, Tineke van den Hoorn,† Wanda van der Stel, Sylvia Le Dévédec, Peter Bouwman,† Jan Willem van der Laan,† and Bob van de Water*†

GFP intensity (a.u.)

- 10 mM E2
- 1 mM E2
- 100 nM E2
- 10 nM E2
- 1 nM E2
- 100 pM E2
- 10 pM E2
- 1 pM E2
- 100 fM E2
- DMSO

00:00 hrs

Fraction of cells (%)
Role of Genomics

- Enhance pharmacological prediction of carcinogenicity
- Proven human non-genotoxic carcinogens:
  - Estrogen hormone and congeners (IARC-list)

Nongenotoxic Carcinogens
Discussion on the mouse in “old S1B”

- Section 6.1 discusses the value of carcinogenicity studies in mice
  - 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.

- 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.

- There is 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.
Continuing discussion on the use of long term mouse studies

Discussion on the mouse in ICH EWG

In negotiations with the EWG is has been decided that a position of a single regulatory authority (EU) should not be included in the Step 4 document. Some examples where a mouse study is not needed are instead included.

Final outcome

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.
2.3 Mouse Carcinogenicity Studies

Additional remarks

There are cases where it may not be appropriate to conduct a mouse carcinogenicity study.

1. 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.

2. 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.
Recent numbers of advices

Over 2021 and 2022
- > 40 cases with Nonclinical Q’s related to Carcinogenicity
- Deferral requested post-approval, with TgRasH2-mice before MAA.
- Claim “no-added value of 2-year rat” as specified in Addendum

Procedural aspect

- SAWP will be the address for requests
- An expert group on Carcinogenicity testing will be involved to ensure continuity and expertise
- The decision will have the character of an advice
- Details to be communicated in the near future.
Carcinogenicity of human pharmaceuticals

Rethinking the Rodent Carcinogenicity testing?

What about the future?
• How to maintain the new S1 approach?
• Should we organize a periodic survey?
• How to organize to exchange the cases?