Using AOPs to Aid Expert Review and Decision-Making in a Weight-of-Evidence Assessment for ICH S1B

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Introduction

The recent addendum to the ICH S1(B) guidance [1] reflects a paradigm shift occurring across industry. The change in approach allows a Weight-of-Evidence (WoE) assessment to be conducted, where multiple pieces of evidence can be collated and assessed against six factors, thus determining if a rat carcinogenicity study is needed or can be waived. Adverse outcome pathways (AOPs) represent an ideal way of organising and contextualising evidence [2] and are a perfect framework for giving transparent, consistent and robust predictions for carcinogenic potential to fulfil the new IC S1B addendum [3].

The transparent contextualisation and, presentation of evidence on an AOP framework not only helps reach automated conclusions but can also be used to aid expert review of such assessments. This system allows for the ability to not only view how data fits together in networks of AOPs, as well as on individual AOPs, but also use knowledge integrated within the AOPs (species relevance, tissue relevance, mechanistic information) to increase confidence in, or mitigate, potential concerns. To illustrate this concept, data has been gathered for Lansoprazole, a known gastric acid inhibitor and an expert review using a carcinogenicity AOP network previously developed [4] conducted to discern if a rat carcinogenicity study is required.

Expert Assessment of Lansoprazole using AOPs

Materials and Methods

Evidence was collected from the following sources:

- Vitic [5]
- Open Targets [6]
- DrugBank [7]
- ToxCast [8]
- ChEMBL [9]
- Drugs@FDA [10]
- Derek Nexus [11]

The evidence was associated to the corresponding key events (KEs) in an AOP network of 37 AOPs for carcinogenicity [4]. An initial overall call was derived from the overall network using the approach described by Stalford et al [3]. The network was then split into six networks based on the AOPs which correspond to the ICH S1B factors. Each network was reviewed to decide if the factor was of carcinogenic concern. In some cases, AOPs were analyzed individually to determine mode-of-action (MoA) and applicability. The combination of these evaluations were then brought together to give an expert-reviewed assessment as to if a rat carcinogenicity study is required.

Initial Overall Call – CONCERN

Organising of the evidence (Table1, Table 2) on the AOP framework showed that an overall call for carcinogenic potential would be positive (Figure 1). The data and the studies used were considered reliable thus there was confidence in the outcome. Initial carcinogenic concern seems to be driven by in vivo histopathological findings in repeat dose studies. Another concern is the positive results for two off-target binding studies, which indicate there may be unintended activity. Therefore expert review is required to confirm the drivers for histopathological findings. An analysis is also required to determine if there is any carcinogenicity-related concern for the intended target.

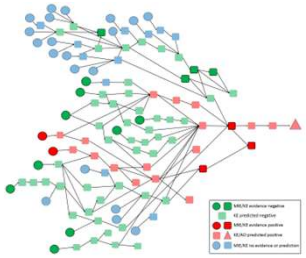

Figure 1: Initial overall call for carcinogenicity based on available evidence

Assay [5,8,9,11]	Outcome
Aryl hydrocarbon receptor binding	Positive
Pregnane X Receptor binding	Positive
Other binding or in silico predictions	Negative
Ames	In silico Negative In vitro Negative
Mouse lymphoma assay	Negative
Chromosome Damage	In vitro Positive In vivo Negative
Micronucleus	In vitro Conflicted In vivo Negative
Unscheduled DNA synthesis	In vitro Negative In vivo Negative

Table 1: off-target binding and genotoxicity data for Lansoprazole

Assay [5,10]	Measure	Tissues (species)
Repeat-Dose Sub-chronic Assay	Hyperplasia	Stomach (rat, dog)
	Hypertrophy	Stomach (rat, dog), liver (rat)
	Organ Weight Increase	Heart (rat), liver (rat, dog), lungs (rat), kidney (rat, dog), ovaries (dog)
Repeat-Dose Chronic Assay	Hyperplasia	Stomach (rat, dog), testes (rat)
	Hypertrophy	Stomach (rat, dog)
	Organ Weight Increase	Stomach (rat)

Table 2: repeat dose sub-chronic and chronic assay findings in rats and dogs

Histopathology Call – Concern

Hyperplasia of the stomach is seen across rats and dogs in sub-chronic and chronic assays (Table 2), which is consistent with carcinogenicity findings for other compounds in this pharmacological class [5,10]. These findings are very likely to drive carcinogenicity in humans and rats. Additionally, hypertrophy is observed in the stomach (rats) and liver (rats), and organ weight increases are seen in multiple tissues in rats and dogs. While these findings are important, they are only indirectly linked to the adverse outcome, meaning they are unlikely to contribute to carcinogenicity without the corresponding hyperplastic findings (Figure 4).

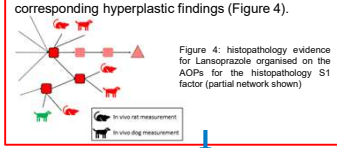

Figure 4: histopathology evidence for Lansoprazole organised on the AOPs for the histopathology S1 factor (partial network shown)

Immunotoxicity Call – No Concern

When analysis of blood chemistry and immunotoxicity-related tissue evidence from the repeat-dose studies [Table 2] is contextualised on the AOP network, it is clear that no MoAs are unaccounted for, thus we can be confident immunotoxicity is unlikely to contribute to carcinogenicity in humans or rats (Figure 2).

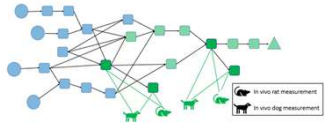

Figure 2: immunotoxicity evidence for Lansoprazole organised on the AOPs for the immunotoxicity S1 factor

Genotoxicity Call – No Concern

Multiple genotoxicity assays have been carried out [Table 1], which would satisfy the requirement for genotoxicity battery testing in ICH S2(R1) [12]. Most results are negative, however two in vitro assays had positive or conflicting findings. However, when contextualised on the AOP network, coverage of the relevant AOPs is good, and it is clear that these results are of no concern, as the equivalent in vivo assays are clearly negative and the WoE approach taken puts confidence in in vivo outcomes over in vitro outcomes (Figure 3). Therefore, it can be concluded that genotoxicity is unlikely to contribute to carcinogenicity in humans or rats.

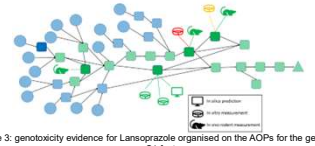

Figure 3: genotoxicity evidence for Lansoprazole organised on the AOPs for the genotoxicity S1 factor

Hormonal Perturbation Call – No Concern in Humans, Possible Concern in Rats

In sub-chronic and chronic studies in rats, some histopathological findings related to endocrine tissues are observed, however findings directly linked to carcinogenicity are only observed in chronic studies [Table 2]. No changes to thyroid hormone levels or copora lutea were observed across all studies conducted [10]. Additionally, endocrine-related findings are not observed in rat carcinogenicity studies for similar compounds in this pharmacological class [5]. The evidence was contextualised based on species (rat v non-rodent) given the differences in observations. When taken into consideration with the binding data [Table 1], all the potential AOPs which could contribute are covered. It is clear from the non-rodent AOP network that hormonal perturbation is unlikely to contribute to carcinogenicity in humans (Figure 5A). For the rat AOP network (Figure 5B) however, while there is a positive binding study for PXR, and the AOP is related to hormonal perturbation, the knowledge embedded within the AOP makes it clear that it is not relevant for the testicular finding. Taking this evidence with the inconsistency between studies and uniqueness of this finding for the drug class, it is possible that hormonal perturbation will contribute to carcinogenicity in rats.

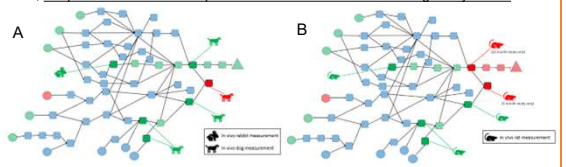

Figure 5: hormonal perturbation evidence for A) non-rodents and B) rats for Lansoprazole organised on the AOPs for the hormonal perturbation S1 factor

Off-Target Call – No Concern

Evidence from binding assays and in silico predictions indicates two potential off-target MoA which may contribute to carcinogenic potential – AhR and PXR (Table 1). These AOPs were examined, combining histopathology and other evidence from sub-chronic and chronic assays. For the AhR AOP, the increase of a hepatic drug-metabolising enzyme [10] supports the the positive binding assay, indicating that the MoA is relevant for Lansoprazole. However, the lack of hyperplasia findings in the liver or lungs in rats and dogs indicate that this MoA is unlikely to contribute to carcinogenicity (Figure 7A). Likewise, for the PXR AOP, the lack of hyperplasia findings in the liver, thyroid or kidneys indicate that a PXR MOA is unlikely to contribute to carcinogenicity. Additionally, differences in Cyp2B activity between rats and dogs [10] indicate that a PXR MoA may be human irrelevant (Figure 7B&C).

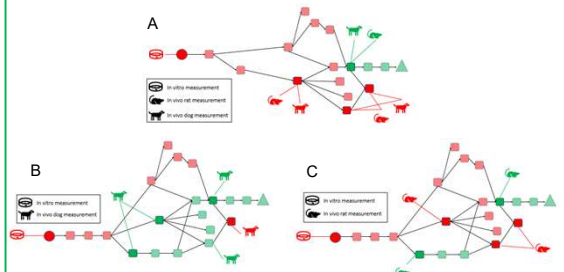

Figure 7: A) evidence on AOP for AhR binding leading to carcinogenicity; C) non-rodent evidence on AOP for PXR binding leading to carcinogenicity; D) rat evidence on AOP for PXR binding leading to carcinogenicity

On-Target Call – Concern

As the target for Lansoprazole is unknown in the AOP network, expert review of the target, the primary pharmacology of the compound, and review of similar compounds was undertaken:

- ATP4A is a catalytic subunit of the gastric H^+/K^+ ATPase pump, transporting ions across the apical membrane of parietal cells [6].
- Primary pharmacologic MoA is to act as a protein pump inhibitor, specifically inhibiting ATPase which transports potassium ions, thus supressing gastric acid production [7].
- ATP4A is homologous across multiple species, including rat, dog, rabbit, mouse and primates [6].
- ATP4A predominantly expressed in the stomach [6].
- Similar compounds which act against the same target include Omeprazole, Rabeprazole and Pantoprazole [6]; **all of these compounds produce stomach tumours in 2-year rat carcinogenicity studies** [5].
- In genetically engineered mice without functioning ATPase, a number of effects are observed when compared to wild –type mice, including **hypergastrinemia**, achlorhydria, metaplasia, dysplasia, and **hyperplasia in stomach tissues** and changes in iron absorption leading to anaemia [6].

The evidence strongly indicates that inhibiting this target with Lansoprazole can lead to carcinogenicity in the stomach in multiple species. This is consistent with the observed histopathology in the stomach in sub-chronic and chronic animal studies. Additionally, inhibition of gastric acid secretion and hypergastraemia and KEs in an AOP with a different MIE relevant to stomach carcinogenesis, therefore the target can be linked in to the AOP network and a new AOP created (Figure 6). This AOP clearly shows that inhibition of ATP4A is very likely to drive carcinogenicity in humans and rats, based on expert review and histopathological data.

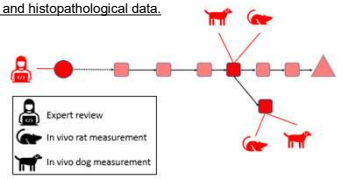

Figure 6: AOP created to describe the on-target inhibition of ATP4A leading to carcinogenesis based on expert review and existing KEs in the AOP network. Histopathological data for the stomach was also added to confirm the primary MoA for carcinogenicity.

Discussion and Conclusions – LIKELY IN HUMANS

The example given illustrates how 1) AOPs are an effective framework for organizing and contextualizing evidence to give a transparent, consistent and robust outcome to aid ICH S1B(R1) WoE decisions, and 2) how AOPs are a vital tool to aid expert review. In this example, organizing and contextualizing available experimental evidence and in silico predictions indicates that the carcinogenic potential of Lansoprazole in humans is **likely, such that a 2-year rat carcinogenicity study would not add value.** Analyzing the results further, by factor and by AOP where required, and then conducting an expert review, considering knowledge embedded within the AOPs for e.g., tissue relevance and considering differences in species outcomes, as well as similar compounds, confirms that the carcinogenic potential of Lansoprazole in humans is likely, and is driven by interaction with the drug target, which **would likely give stomach tumours in rats and humans, and possibly additional testicular tumours in rats only**. Examination of 2-year rat carcinogenicity data indicates that the outcome using AOPs is accurate, with studies showing tumours in both the stomach and testes [5,10].* Therefore, if this was a new drug coming to market, there would be no need to conduct a 2-year rat carcinogenicity study, saving time and animals.

*It is worth noting that, while in this illustrative example, and in known studies, Lansoprazole has been shown to carcinogenic, it is widely used to treat gastric ulcers and reflux diseases. So, is it safe to use? The answer is yes! ICH S1B(R1) is used to assess the hazard, but not necessarily the risk of carcinogenicity. Based on the intended dose and recommended period of usage for these drugs, it is unlikely that histopathological changes in the stomach will occur based on the exposure . Additionally, studies with genetically engineered mice lacking ATPase and subchronic/chronic rodent studies with recovery periods show that the effects are reversible when an acidic environment in the stomach is (re)introduced [6,10].

References:

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