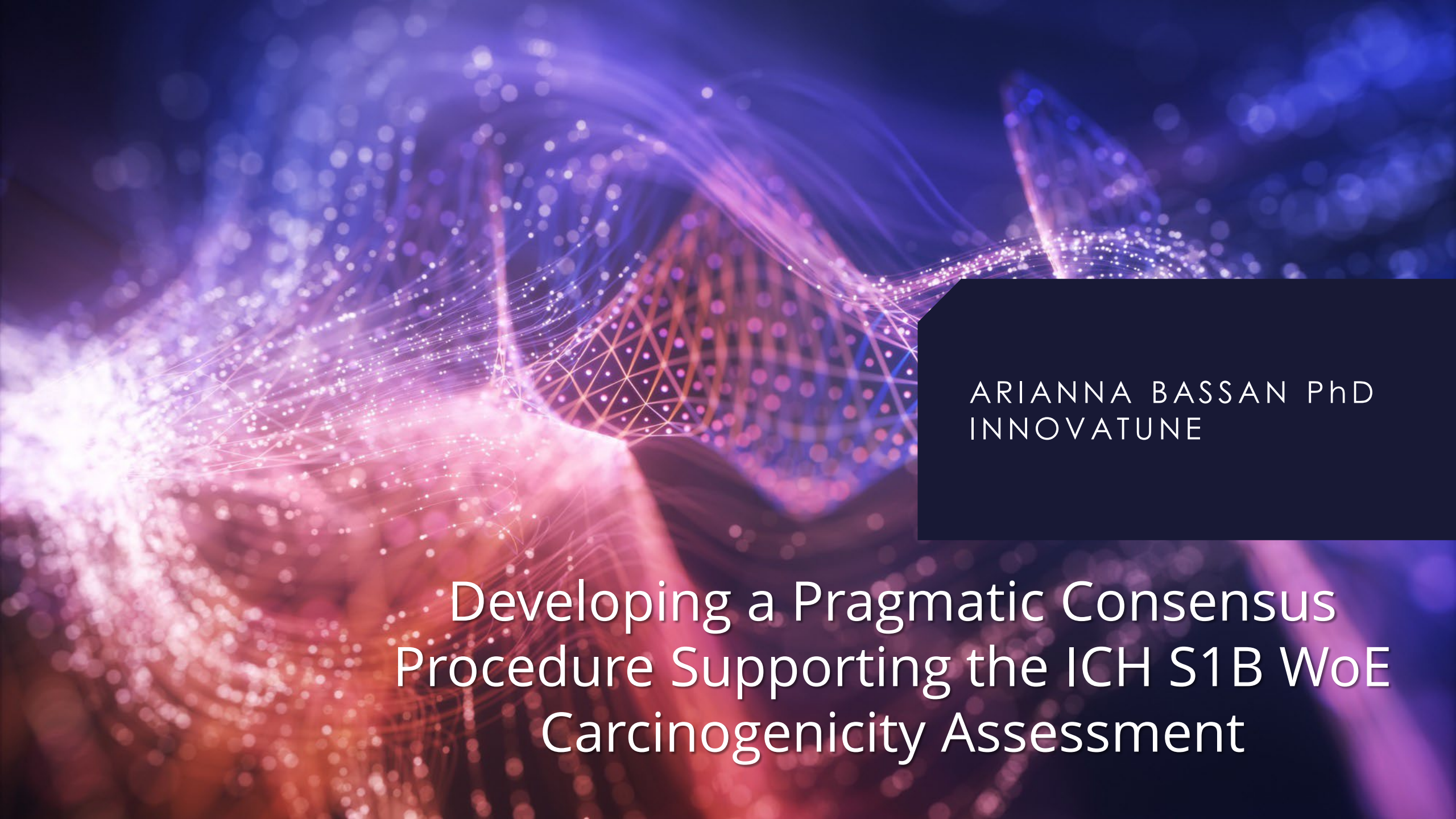

ARIANNA BASSAN PhD
INNOVATUNE

Developing a Pragmatic Consensus Procedure Supporting the ICH S1B WoE Carcinogenicity Assessment

THIS PRESENTATION IS ABOUT …

… ongoing effort of a **working group** to establish **a pragmatic consensus procedure** that brings together key information to support the ICH S1B WoE carcinogenicity assessment

… this effort aims at **standardizing a procedure** that frames the ICH S1B human carcinogenicity assessment, tracking properties and effects that drive the outcome of the assessment

INTERNATIONAL WORKING GROUP

- Initiative **promoted and supported by INSTEM**

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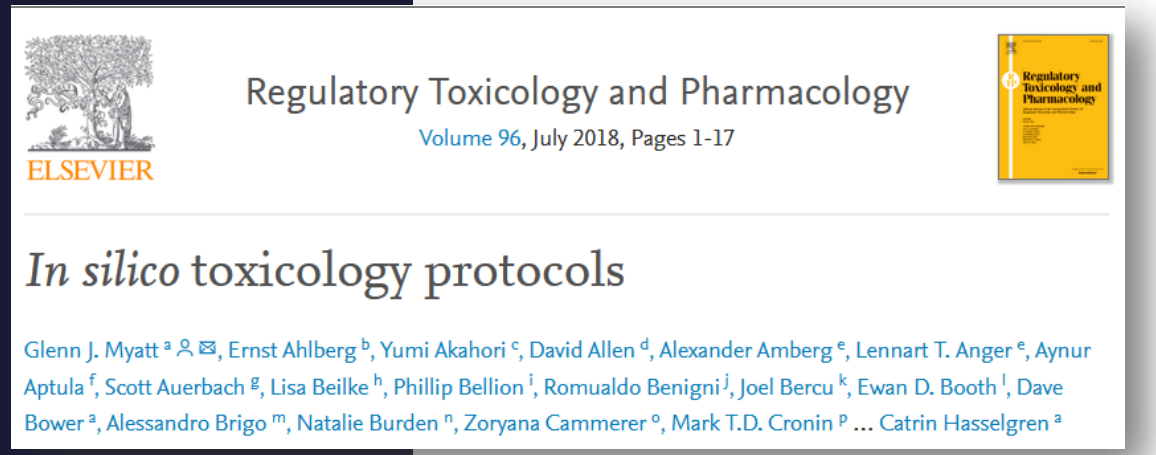
- Experts from different organizations have joined in the effort to establish **a pragmatic consensus procedure** supporting the ICH S1B WoE carcinogenicity assessment

- **Coordination of the working group**: Arianna Bassan

BUILDING ON PREVIOUS WORK

- The *in silico* **toxicology protocols initiative**
  - International cross-industry consortium comprised of different organizations
  - Combining evidence as coming from different sources (e.g., in vitro and in vivo experimental data and in silico results) to establish an overall assessment and confidence score for a given toxicological endpoint
  - A protocol helps ensure any assessment is performed in a transparent, accepted, consistent, documented, and repeatable manner

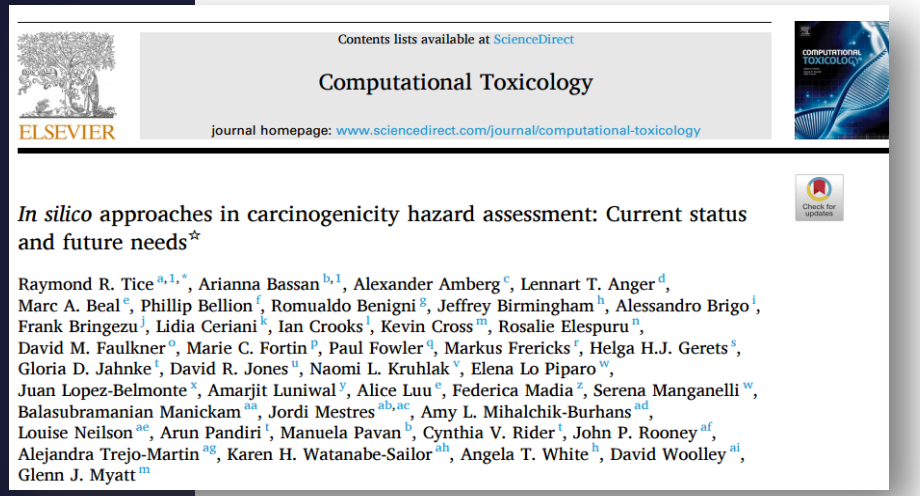
- The *in silico* toxicology protocol approach is applied in more general terms to the ICH S1B WoE assessment
  - Endpoint of interest: added value of a two-year rat study to the assessment of human carcinogenic risk



RECENT CANCER ACTIVITIES

- *In silico* approaches in carcinogenicity hazard assessment: Current status and future needs

- *In Silico* Approaches in Carcinogenicity Hazard Assessment: Case Study of Pregabalin, a Nongenotoxic Mouse Carcinogen



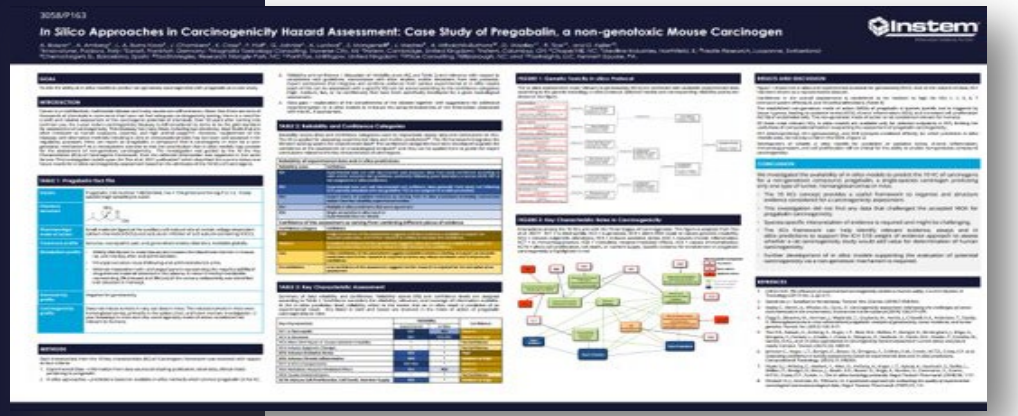
Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.sciencedirect.com/journal/computational-toxicology

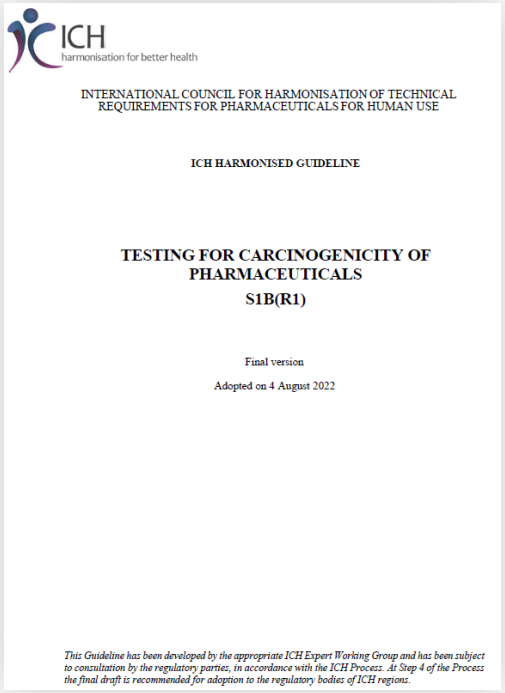
In silico approaches in carcinogenicity hazard assessment: Current status and future needs☆

Raymond R. Tice [a,1,*], Arianna Bassan [b,1], Alexander Amberg [c], Lennart T. Anger [d], Marc A. Beal [e], Phillip Bellion [f], Romualdo Benigni [g], Jeffrey Birmingham [h], Alessandro Brigo [i], Frank Bringezu [j], Lidia Ceriani [k], Ian Crooks [l], Kevin Cross [m], Rosalie Elespuru [n], David M. Faulkner [o], Marie C. Fortin [p], Paul Fowler [q], Markus Frericks [r], Helga H.J. Gerets [s], Gloria D. Jahnke [t], David R. Jones [u], Naomi L. Kruhlak [v], Elena Lo Piparo [w], Juan Lopez-Belmonte [x], Amarjit Luniwal [y], Alice Luu [e], Federica Madia [z], Serena Manganelli [w], Balasubramanian Manickam [aa], Jordi Mestres [ab,ac], Amy L. Mihalchik-Burhans [ad], Louise Neilson [ae], Arun Pandiri [t], Manuela Pavan [b], Cynthia V. Rider [t], John P. Rooney [af], Alejandra Trejo-Martin [ag], Karen H. Watanabe-Sailor [ah], Angela T. White [h], David Woolley [ai], Glenn J. Myatt [m]



In Silico Approaches in Carcinogenicity Hazard Assessment: Case Study of Pregabalin, a non-genotoxic Mouse Carcinogen

ICH S1B GUIDELINE



ICH
harmonisation for better health

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

ICH HARMONISED GUIDELINE

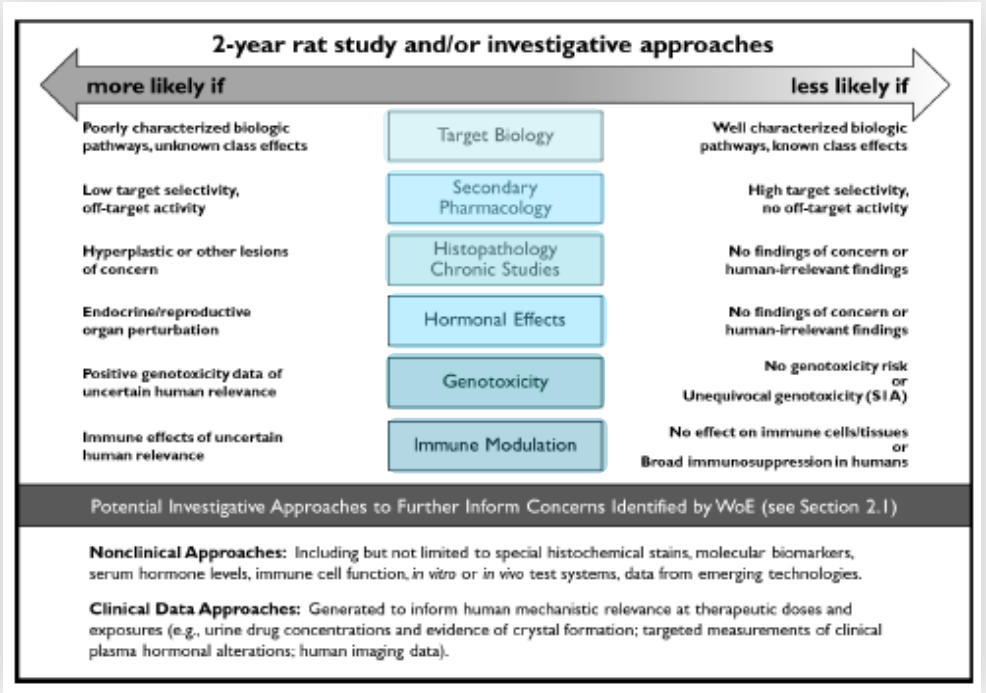
TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS
S1B(R1)

Final version

Adopted on 4 August 2022

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of ICH regions.

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation



2-year rat study and/or investigative approaches

more likely if		less likely if
Poorly characterized biologic pathways, unknown class effects	Target Biology	Well characterized biologic pathways, known class effects
Low target selectivity, off-target activity	Secondary Pharmacology	High target selectivity, no off-target activity
Hyperplastic or other lesions of concern	Histopathology Chronic Studies	No findings of concern or human-irrelevant findings
Endocrine/reproductive organ perturbation	Hormonal Effects	No findings of concern or human-irrelevant findings
Positive genotoxicity data of uncertain human relevance	Genotoxicity	No genotoxicity risk or Unequivocal genotoxicity (S1A)
Immune effects of uncertain human relevance	Immune Modulation	No effect on immune cells/tissues or Broad immunosuppression in humans

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).

There is no "one size fits all" approach for the novel strategy described in the ICH S1B addendum and its application must be tailored to the specific pharmaceutical being evaluated.

AIM AND EXPECTED RESULTS OF THE WORKING GROUP

AIM

To pragmatically **standardize a procedure** that frames the ICH S1B human carcinogenicity assessment ensuring as much as possible that any assessment is performed in a transparent, consistent, documented, repeatable, and defendable manner

RESULT

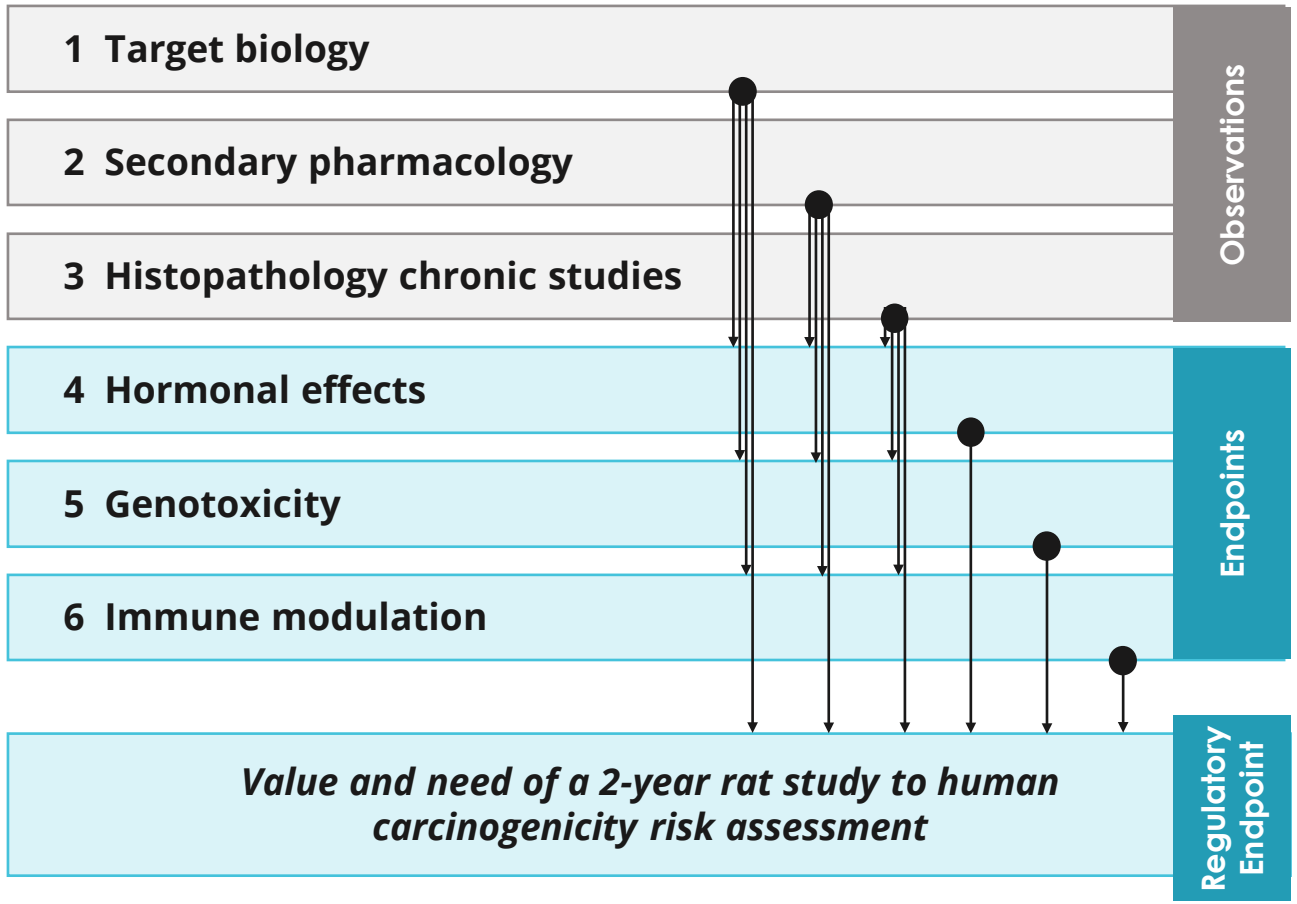
Pragmatic consensus procedure that serves:

1. as a **guide to organizing the studies** and displaying the data in the proper format
2. to **clarify what would be expected in terms of the types of integrated evidence** to be presented in the Carcinogenicity Assessment Document

APPROACH TO ESTABLISH THE PRAGMATIC PROCEDURE

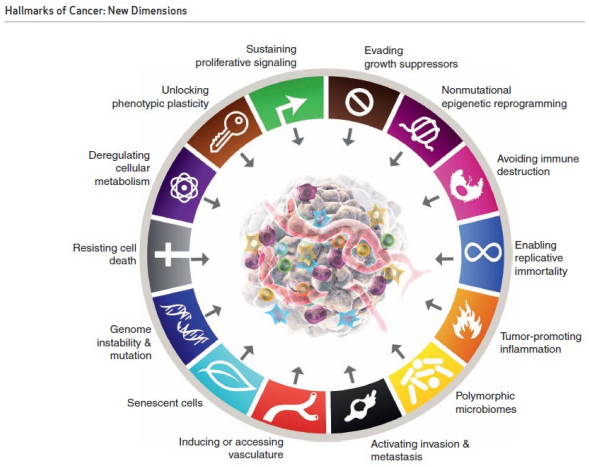
- **KEY EVIDENCE** linked to the WoE criteria needs to be identified along with the sources of such information (especially when coming from novel investigative approaches).

- **REPORTING FORMAT** of data, results, and conclusions is to be defined to clarify what is expected in terms of the types of evidence to be included and critical questions to be answered.

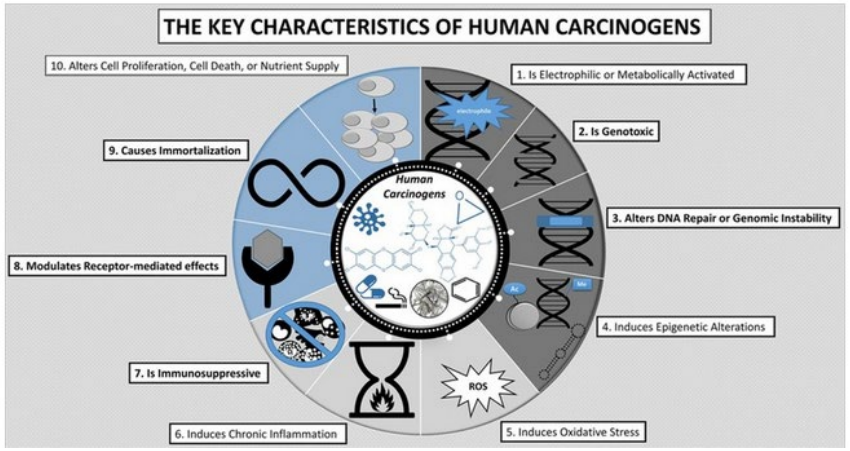


CONCEPTUAL FRAMEWORKS

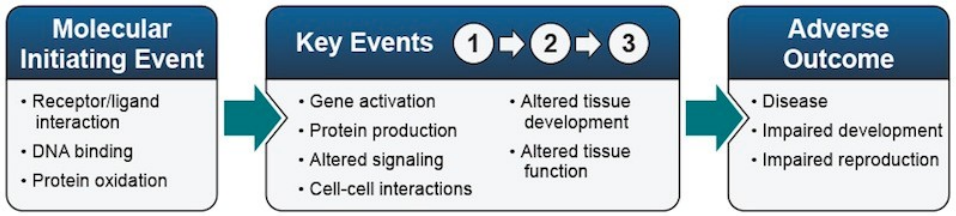
Different conceptual frameworks can support the identification of more granular information related to the ICH S1B WoE criteria



Hanahan Cancer Discovery, 2022



Guyton et al Chem Res Toxicol, 2018



https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-aop/aop.html

- **Hallmarks of cancer**
  - To recapitulate the **functional capabilities of cells** collectively leading to malignant growth
- **10 key characteristics of carcinogens**
  - To summarize **properties of chemicals** contributing to carcinogenesis.
- **AOPs**
  - To connect sequential chain of causally linked events (MIE and KEs) to a clinically relevant health effect.

WoE FACTORS

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation

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TARGET BIOLOGY FACTOR FROM THE ICH S1B ADDENDUM

Description	2-year rat study and/or investigative approaches …	
	more likely if …	less likely if …
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,	"Poorly characterized biologic pathways, unknown class effects"	"Well characterized biologic pathways, known class effects"

KEYWORDS

- DRUG TARGET BIOLOGY
- PRIMARY PHARMACOLOGIC MECHANISM
- DRUG TARGET DISTRIBUTION
- PHARMACOLOGIC ACTIVITY AND POTENCY
- GENETICALLY ENGINEERED MODELS
- HUMAN GENETIC ASSOCIATION STUDIES
- CANCER GENE DATABASES
- CLASS EFFECTS
- MAJOR METABOLITES

Relevant outcome generally supporting no value of the 2-year rat study

- Target biology not associated with **cellular pathways** known to be involved with human cancer development
- **Non-mammalian** pharmaceutical target (e.g., viral, microbial)
- No compound-related carcinogenicity findings conducted in compounds with the **same pharmacologic drug class**
- No findings related to carcinogenicity in **knock-out mice** (drug target)

TARGET BIOLOGY FACTOR – REPORT STANDARDIZATION

The target biology evaluation should use a repeatable, unbiased, and extensive analysis of the literature and relevant biological databases

Report section	Content
Materials and methods	Description of the **different databases** (including version numbers), the **searches** (and date performed) and any other data science procedures (data analysis, artificial intelligence, machine learning, data processing, modelling, etc) that were used to generate the raw or processed output.
Drug target pharmacology	Background information on **the pharmacological activity of the pharmaceutical**, and any known human metabolites and their target(s).
Carcinogenicity assessment of primary pharmacological class	This section would identify, summarize, and assess the human relevance of carcinogenicity data for other drugs in the **same pharmacological class**.
Summary of target pathway(s) and cancer risk	This section may include general background biology information summarizing **the normal physiological role of the target**. An assessment of **how well the primary pharmacology pathway is characterized** should be performed based on an expert review of the completeness of the target pathway(s) information. An analysis of these target pathways may be conducted to understand the plausibility of any direct gene associations to tumor development.

WoE FACTORS

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation

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SECONDARY PHARMACOLOGY FACTOR FROM THE ICH S1B ADDENDUM

Description	2-year rat study and/or investigative approaches … more likely if …	2-year rat study and/or investigative approaches … less likely if …
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: 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),	"Low target selectivity, off-target activity"	"High target selectivity, no off-target activity"

KEYWORDS

- SCREENS
- SELECTIVITY and OFF-TARGET POTENTIAL
- SCREENS THAT INFORM CARCINOGENIC RISK
- (parent compound and major metabolites)

Relevant outcome generally supporting no value of the 2-year rat study

- High target selectivity when compared with other targets
- No evidence of off-target interactions at drug concentrations up to 10 µM, including no interaction with estrogen, androgen, glucocorticoid receptors
- **Known pharmacology of off-target receptors** not associated with tumorigenesis

SECONDARY PHARMACOLOGY – CANCER-RELATED TARGETS?

- **ICH S1B assessment:** general promiscuity with focus on specific interaction with **cancer-relevant targets**
  - Information on cancer-relevant targets is sparse in the literature.
- The working group has performed an investigation of the association between targets and cancer-relevant pathways
  - Associations between MIEs/KEs and cancer-related targets identified in the AOP Wiki:

List of **580 targets** from available off-target batteries used by Industry (including kinases, GPCRs, ion channels and nuclear hormone receptors)

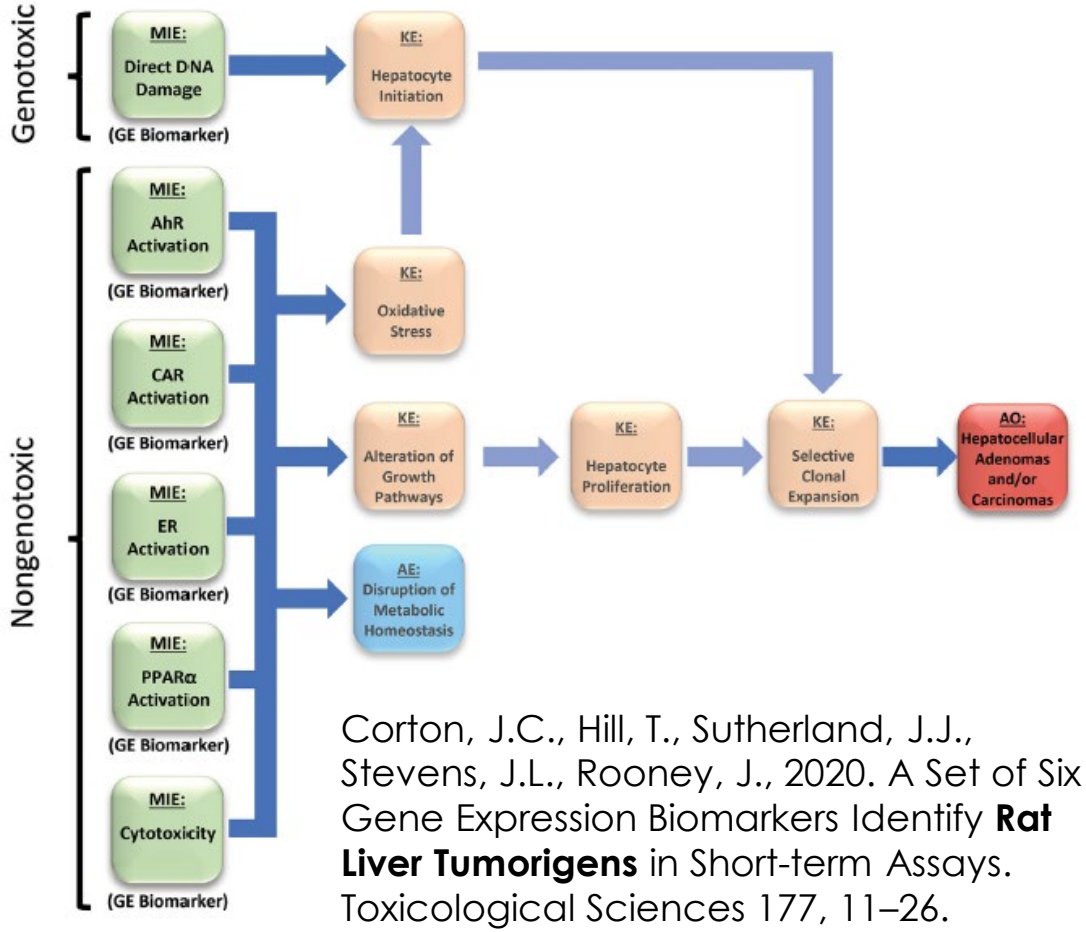
Search of cancer–related AOPs in AOPWiki

21 targets have an '**association**' with cancer related AOPs

***AOP Knowledgebase**, 2022. AOPwiki [WWW Document]. URL https://aopwiki.org/*

SECONDARY PHARMACOLOGY WoE CONSIDERATIONS

- Cancer-related targets
  - Associations versus demonstrated/plausible relationship
  - Target biology approach may be used to get further insights on “associations”



Corton, J.C., Hill, T., Sutherland, J.J., Stevens, J.L., Rooney, J., 2020. A Set of Six Gene Expression Biomarkers Identify **Rat Liver Tumorigens** in Short-term Assays. Toxicological Sciences 177, 11–26.

WoE FACTORS

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation

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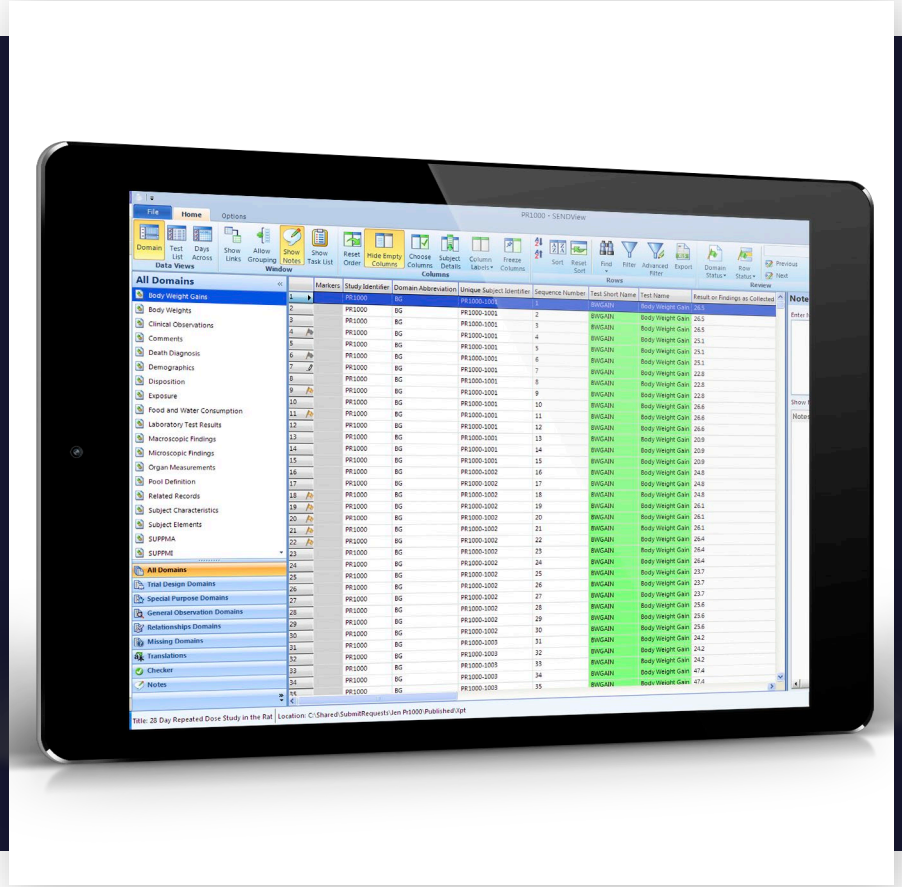
HISTOPATHOLOGY WoE FACTOR

- Histopathology chronic studies
  - Detailed description of relevant signals in the ICH S1B guidance
  - Efficient use of dictionary of terms to report histopathological findings
    - Comprehensive list of potential observations that can be cross-checked based on results.



WoE FACTORS

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation

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GENOTOXICITY WoE FACTOR

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,

From the guidance

- Reference to existing protocol (based on ICH S2) and possible refinement (e.g., incorporation of data generated by technologies such **transcriptomics** or high throughput screening assays to support the decision process)

WoE FACTORS

1. Target biology
2. Secondary pharmacology
3. Histopathology
4. Hormonal perturbation
5. Genetic toxicology
6. Immune modulation

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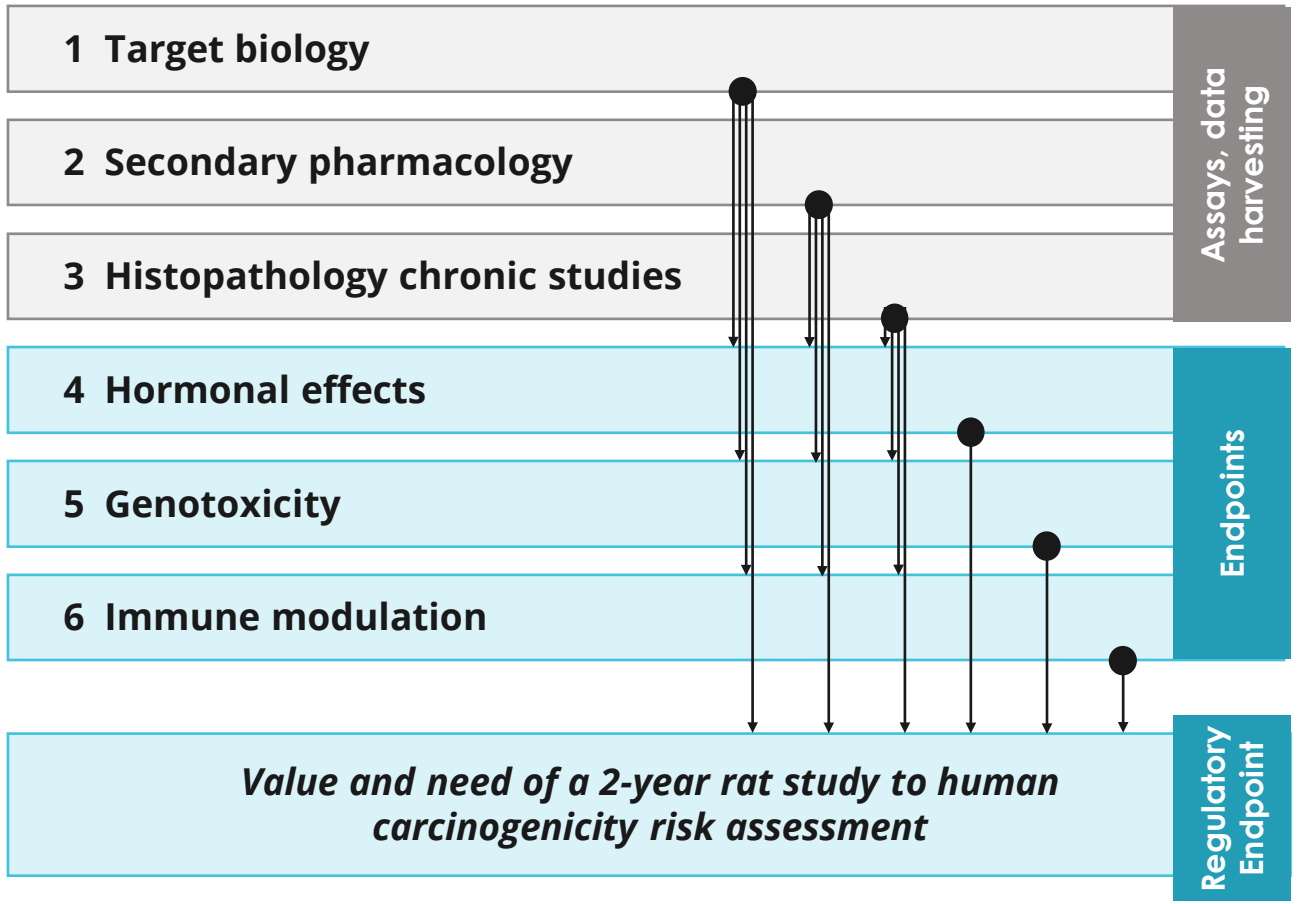
HORMONAL PERTURBATION AND IMMUNOMODULATION WoE FACTORS

- Hormonal perturbation
  - Detailed description of relevant signals in the ICH S1B guideline
    - Main input: histopathological observations
    - Input from target biology
- Immune modulation
  - From ICH S1B guideline: evidence of immune modulation in accordance with ICH S8 guideline
    - Challenging endpoint



CHALLENGES AND PERSPECTIVES

Pragmatic approach	Identify **relevant** evidence (i.e., cancer-related) from current core approaches	**Associations** versus demonstrated relationships (cause-effect)?
Consistency	A **consensus procedure** that guides the integration of relevant information	How to develop a procedure that stands the **test of time**? Which **additional investigative approaches** to sustain an intelligent decision making?
Confidence	Provide the assessment with an evaluation of **confidence**	How to develop a **decision support system** accounting for the complexity of weight of the evidence?
Timing	**Early planning** of the integrative WoE carcinogenicity assessment	Timing of submission
Regulatory endpoint	*Would the rat carc study add value?*	Complexity in assessing human-relevant evidence

ACKNOWLEDGEMENTS

COLLABORATORS ON THE ICH S1B WORKING GROUP:

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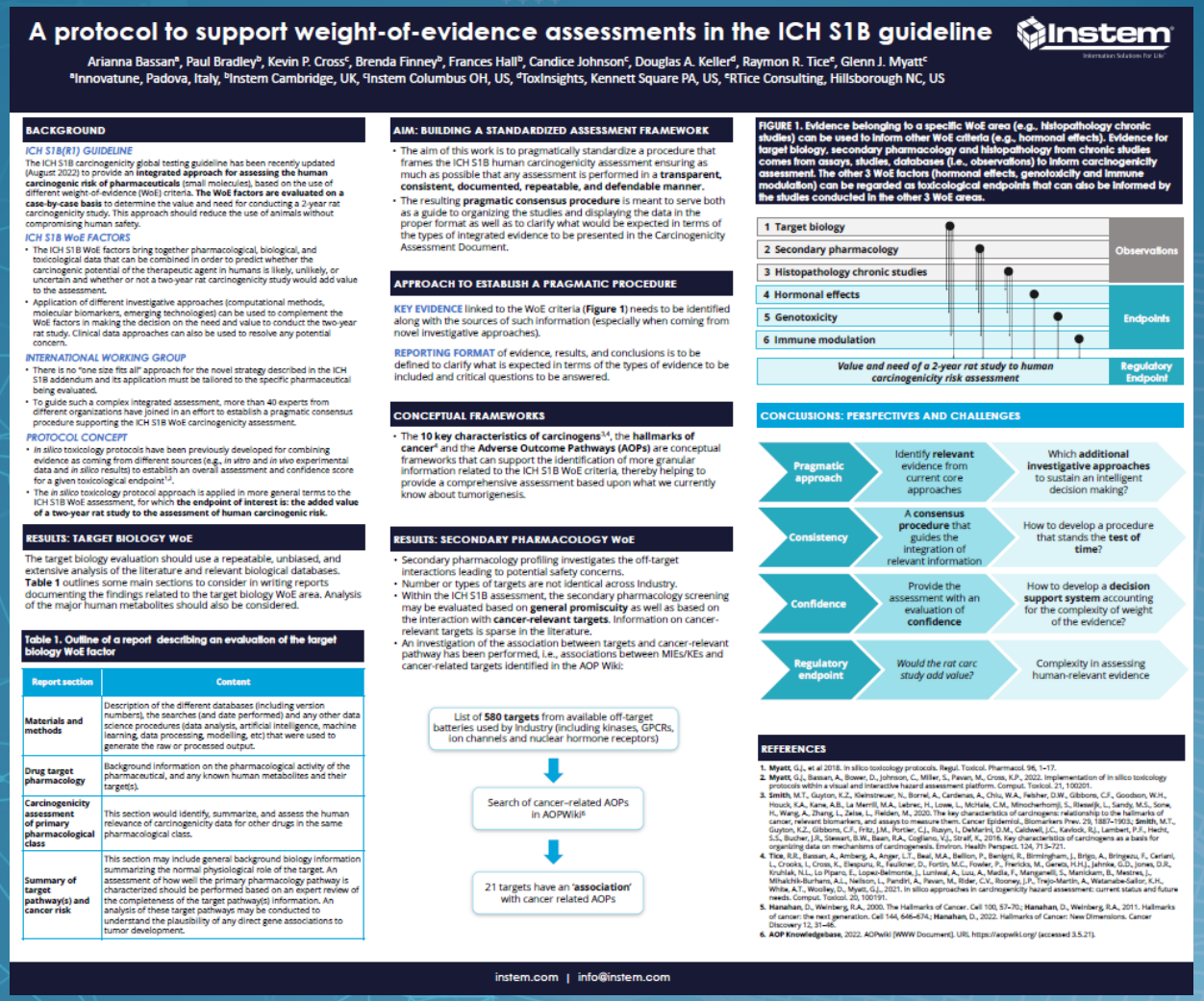
A Protocol To Support Weight-of-Evidence Assessments In The ICH S1B Guideline

Arianna Bassan[a], Paul Bradley[b], Jon Chambers[b], Kevin P. Cross[c], Brenda Finney[b], Frances Hall[b], Candice Johnson[c], Douglas A. Keller[d], Raymond R. Tice[e], Glenn J. Myatt[c]
[a]Innovatune, Padova, Italy, [b]Instem Cambridge, UK, [c]Instem Columbus OH, US, [d]ToxInsights, Kennett Square PA, US, [e]RTice Consulting, Hillsborough NC, US



A protocol to support weight-of-evidence assessments in the ICH S1B guideline

Arianna Bassan[a], Paul Bradley[b], Kevin P. Cross[c], Brenda Finney[b], Frances Hall[b], Candice Johnson[c], Douglas A. Keller[d], Raymon R. Tice[e], Glenn J. Myatt[c]
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BACKGROUND

ICH S1B(R1) GUIDELINE

The ICH S1B carcinogenicity global testing guideline has been recently updated (August 2022) to provide an **integrated approach for assessing the human carcinogenic risk of pharmaceuticals** (small molecules), based on the use of different weight-of-evidence (WoE) criteria. **The WoE factors are evaluated on a case-by-case basis** to determine the value and need for conducting a 2-year rat carcinogenicity study. This approach should reduce the use of animals without compromising human safety.

ICH S1B WoE FACTORS

- The ICH S1B WoE factors bring together pharmacological, biological, and toxicological data that can be combined in order to predict whether the carcinogenic potential of the therapeutic agent in humans is likely, unlikely, or uncertain and whether or not a two-year rat carcinogenicity study would add value to the assessment.
- Application of different investigative approaches (computational methods, molecular biomarkers, emerging technologies) can be used to complement the WoE factors in making the decision on the need and value to conduct the two-year rat study. Clinical data approaches can also be used to resolve any potential concern.

INTERNATIONAL WORKING GROUP

- There is no "one size fits all" approach for the novel strategy described in the ICH S1B addendum and its application must be tailored to the specific pharmaceutical being evaluated.
- To guide such a complex integrated assessment, more than 40 experts from different organizations have joined in an effort to establish a pragmatic consensus procedure supporting the ICH S1B WoE carcinogenicity assessment.

PROTOCOL CONCEPT

- *In silico* toxicology protocols have been previously developed for combining evidence as coming from different sources (e.g., *in vitro* and *in vivo* experimental data and *in silico* results) to establish an overall assessment and confidence score for a given toxicological endpoint[1,2].
- The *in silico* toxicology protocol approach is applied in more general terms to the ICH S1B WoE assessment, for which **the endpoint of interest is: the added value of a two-year rat study to the assessment of human carcinogenic risk.**

RESULTS: TARGET BIOLOGY WoE

The target biology evaluation should use a repeatable, unbiased, and extensive analysis of the literature and relevant biological databases. **Table 1** outlines some main sections to consider in writing reports documenting the findings related to the target biology WoE area. Analysis of the major human metabolites should also be considered.

Table 1. Outline of a report describing an evaluation of the target biology WoE factor

Report section	Content
Materials and methods	Description of the different databases (including version numbers), the searches (and date performed) and any other data science procedures (data analysis, artificial intelligence, machine learning, data processing, modelling, etc) that were used to generate the raw or processed output.
Drug target pharmacology	Background information on the pharmacological activity of the pharmaceutical, and any known human metabolites and their target(s).
Carcinogenicity assessment of primary pharmacological class	This section would identify, summarize, and assess the human relevance of carcinogenicity data for other drugs in the same pharmacological class.
Summary of target pathway(s) and cancer risk	This section may include general background biology information summarizing the normal physiological role of the target. An assessment of how well the primary pharmacology pathway is characterized should be performed based on an expert review of the completeness of the target pathway(s) information. An analysis of these target pathways may be conducted to understand the plausibility of any direct gene associations to tumor development.

AIM: BUILDING A STANDARDIZED ASSESSMENT FRAMEWORK

- The aim of this work is to pragmatically standardize a procedure that frames the ICH S1B human carcinogenicity assessment ensuring as much as possible that any assessment is performed in a **transparent, consistent, documented, repeatable, and defendable manner.**
- The resulting **pragmatic consensus procedure** is meant to serve both as a guide to organizing the studies and displaying the data in the proper format as well as to clarify what would be expected in terms of the types of integrated evidence to be presented in the Carcinogenicity Assessment Document.

APPROACH TO ESTABLISH A PRAGMATIC PROCEDURE

KEY EVIDENCE linked to the WoE criteria (**Figure 1**) needs to be identified along with the sources of such information (especially when coming from novel investigative approaches).

REPORTING FORMAT of evidence, results, and conclusions is to be defined to clarify what is expected in terms of the types of evidence to be included and critical questions to be answered.

CONCEPTUAL FRAMEWORKS

- The **10 key characteristics of carcinogens**[3,4], the **hallmarks of cancer**[4] and the **Adverse Outcome Pathways (AOPs)** are conceptual frameworks that can support the identification of more granular information related to the ICH S1B WoE criteria, thereby helping to provide a comprehensive assessment based upon what we currently know about tumorigenesis.

RESULTS: SECONDARY PHARMACOLOGY WoE

- Secondary pharmacology profiling investigates the off-target interactions leading to potential safety concerns.
- Number or types of targets are not identical across Industry.
- Within the ICH S1B assessment, the secondary pharmacology screening may be evaluated based on **general promiscuity** as well as based on the interaction with **cancer-relevant targets**. Information on cancer-relevant targets is sparse in the literature.
- An investigation of the association between targets and cancer-relevant pathway has been performed, i.e., associations between MIEs/KEs and cancer-related targets identified in the AOP Wiki:

List of **580 targets** from available off-target batteries used by Industry (including kinases, GPCRs, ion channels and nuclear hormone receptors)

↓

Search of cancer-related AOPs in AOPWiki[6]

↓

21 targets have an '**association**' with cancer related AOPs

FIGURE 1. Evidence belonging to a specific WoE area (e.g., histopathology chronic studies) can be used to inform other WoE criteria (e.g., hormonal effects). Evidence for target biology, secondary pharmacology and histopathology from chronic studies comes from assays, studies, databases (i.e., observations) to inform carcinogenicity assessment. The other 3 WoE factors (hormonal effects, genotoxicity and immune modulation) can be regarded as toxicological endpoints that can also be informed by the studies conducted in the other 3 WoE areas.

CONCLUSIONS: PERSPECTIVES AND CHALLENGES

Pragmatic approach	Identify **relevant** evidence from current core approaches	Which **additional investigative approaches** to sustain an intelligent decision making?
Consistency	A **consensus procedure** that guides the integration of relevant information	How to develop a procedure that stands the **test of time**?
Confidence	Provide the assessment with an evaluation of **confidence**	How to develop a **decision support system** accounting for the complexity of weight of the evidence?
Regulatory endpoint	*Would the rat carc study add value?*	Complexity in assessing human-relevant evidence

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