Pig-a In Vivo Mutation Assay
Workgroup Report
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Presented By:
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Workgroup

• Chair: Bhaskar Gollapudi, Exponent, Inc.
• Co-chair: Anthony Lynch, GlaxoSmithKline
• Rapporteur: Robert Heflich, US FDA
• Invited Participant: Jennifer Tanir, ILSI-HESI
Workgroup Charter

• To reach consensus on:
  – Assay Maturity
  – Study Design
  – Data Evaluation
  – Strategic Placement of the Assay
  – Future Research Opportunities

• Context:
  – Hazard and risk assessment
Pig-a Assay: Introduction

• *Pig-a* = phosphatidylinositol glycan complementation group A gene

• Gene product is required in the first step of glycophosphatidylinositol (GPI) anchor synthesis
  – GPI anchors attach several proteins to the cell surface of RBCs, e.g. CD59, CD24

• Of the genes required to form GPI anchors, only *Pig-a* is located on the X-chromosome
  – Meaning “one hit” can produce a mutant cell surface phenotype
  – The resulting phenotype can be assessed with antibodies and flow cytometry
Pig-a Cartoon

Wild Type

Cell Surface Protein

CD59

EtN P Man P EtN

Man

GPI Anchor

Plasma membrane

Pig-a Mutation Results in a Cell Surface Phenotype

Figure adapted from Dobrovolsky et al., Environ Mol Mutagen, 51 (2010) 825-835
Assay Maturity

- **Nomenclature**: Assay for ‘GPI-anchored-protein-deficient erythrocytes’ or ‘Pig-a mutant phenotype’.

- **Genetic confirmation**: good supporting evidence.

- **Agents tested**: 26 Ames positives and limited number of non-mutagens from a wide range of chemical space, including those requiring metabolic activation.

- **Intra and inter-laboratory reproducibility**: Good

- **Application**: Recommended as a component in safety assessment
Study Design

- **Species:** *Rats*
- **Number:** 5-6 males; females for sex-specific agents
- **Pre-screening:** Optional, but useful
- **Treatment duration:** 28-day; acute (1-3 days) acceptable if justified.
- **Age, Route, and MTD:** Similar to other related OECD Guidelines.
- **Concurrent positive control:** Not mandatory
- **Blood sampling:** 28 d after treatment initiation; optional earlier and later samples.
Study Design (contd.)

- **Sample storage and shipping:** Overnight shipment and storage up to 5 days acceptable.
- **Staining/analysis:** Both published methods (Litron and Japanese) equally acceptable.
- **Cells analyzed:** Reticulocytes and RBCs.
- **Number of cells:** Minimum 1 to 5 x $10^6$; larger if feasible.
- **Mutant fractions in controls:** Laboratory specific based on accepted quality control methods.
Data Evaluation

• **Statistical methods**: appropriate methods (e.g., $\log_{10}$ transformation MPF followed by ANOVA and pair-wise)
  – RET and RBCs MPF analyzed separately.

• **Data Interpretation**:
  – Biological relevance + appropriate statistical methods
  – Positive Response: Dose-related increase, or clear increase at a single dose.
  – Biological Relevance:
    • ↑ MPF both RETs and RBCs, and at multiple sampling points,
    • Comparison with the historical control distribution
  – Tissue exposure to be confirmed for negative calls.
  – Results not meeting positive or negative criteria = equivocal.
Strategic Placement of the Assay

• *With adequate demonstration of exposure of the bone marrow (or plasma), the assay should be considered*
  • As follow-up of Ames and in vitro mammalian cell gene mutation positives, whether or not they require metabolic activation
  • As an endpoint to build weight of evidence on the in vivo mutagenicity (or lack thereof)
• *Not recommended as follow-up to either in vitro clastogenicity or aneugenicity findings*
Concluding Statements

• The consensus statements and protocol developed by the IWGT workgroup will be published in the coming months.
• This publication could serve as a guidance document for those trying to implement the assay.
• There is optimism for an OECD guideline in the future.
Future Research Opportunities

• Further efforts to confirm genotype.
• Effect of methylation/demethylation on assay performance.
• Additional test substances, especially those not expected to induce an in vivo mutagenic response.
• Examine influence of longer treatments, e.g. >90 days.
• Standardize protocols for other species (e.g., human, mouse, dog, swine) and other haematopoietic cell types.
• Explore utility other tissues, e.g. Liver, germ cells.
• Develop analogous *in vitro* cell culture based assay.
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QUESTIONS??