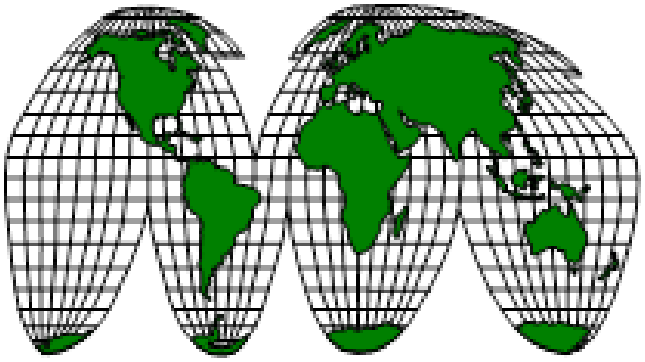

Pig-a *In Vivo* Mutation Assay Workgroup Report

6th International Workshop on Genotoxicity Testing

Presented By:

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Exponent, Inc.

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Workgroup

- **Chair**: *Bhaskar Gollapudi, Exponent, Inc.*
- **Co-chair**: *Anthony Lynch, GlaxoSmithKline*
- **Rapporteur**: *Robert Heflich, US FDA*
- **Members**: *S. Dertinger, V. Dobrovolsky, R. Frotschl, K. Horibata, D. Jacobson-Kram, M. Kenyon, T. Kimoto, D. Lovell, L. Stankowski, P. White, K. Witt*
- **Invited Participant**: *Jennifer Tanir, ILSI-HESI*

Workgroup Charter

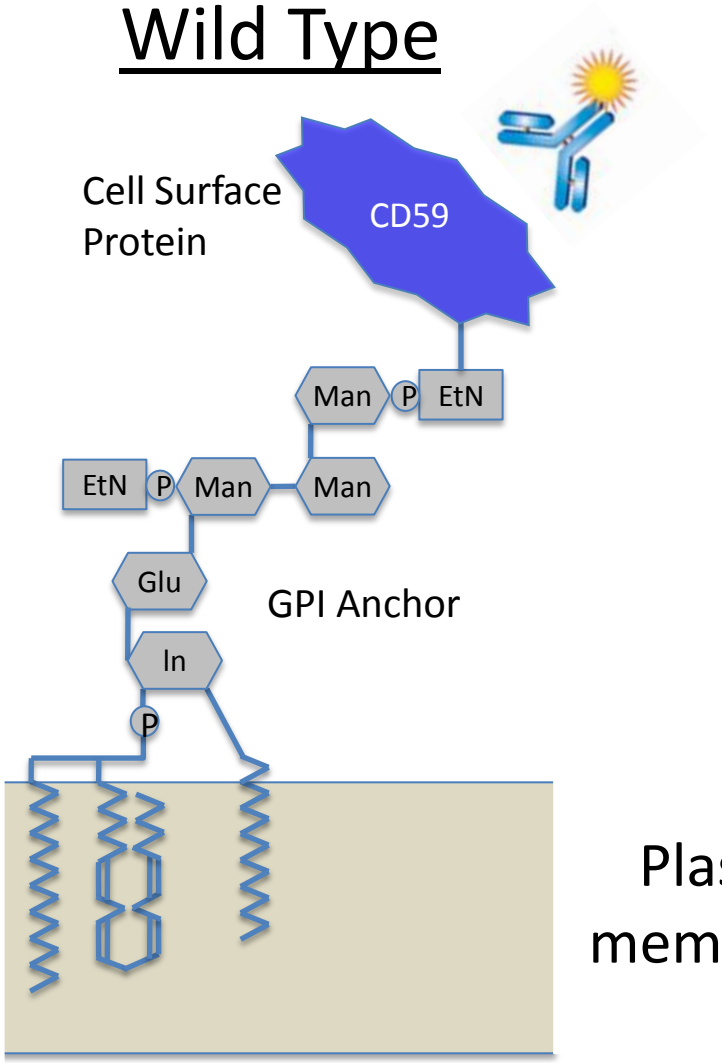
- To reach consensus on:
  - Assay Maturity
  - Study Design
  - Data Evaluation
  - Strategic Placement of the Assay
  - Future Research Opportunities
- Context:
  - Hazard and risk assessment

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



Pig-a Mutation Results in a Cell Surface Phenotype

Plasma membrane

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 relevence + 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 haematopoietc cell types.
- Explore utility other tissues, e.g. Liver, germ cells.
- Develop analogous *in vitro* cell culture based assay.

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Thank you!!

QUESTIONS??