New Technologies for Predicting Genotoxic Risk in Humans

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David Jacobson-Kram, Ph.D., DABT
Office of New Drugs, CDER, FDA
Role of genetic toxicology testing

Genetic toxicology data serve different purposes:

- In drug development place holder for carcinogenic risk until results of carcinogenicity studies are received.
- For most new chemicals, short-term predictors of long-term risk.
- Useful in interpreting MoA in positive carcinogenicity studies.
ICH guidance specifies which genetox studies should be performed on a new drug and provides guidance on how the tests should be performed

- S2A Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals
- S2B A Standard Battery of Genotoxicity Testing for Pharmaceuticals
- Published in 1995 and 1997
S2B* A Standard Battery of Genotoxicity Testing for Pharmaceuticals

- Bacterial reverse mutation assay (Ames test)
- An *in vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an *in vitro* mouse lymphoma tk assay
- An *in vivo* test for chromosomal damage using rodent hematopoietic cells (micronucleus assay or chromosome aberration test).

* before revision process
Positive results

“positive result in vitro [almost always in mammalian cell assays] is followed up by a second in vivo study--using tissue other than bone marrow.” Generally, rat liver UDS.
ICH S2A and S2B: Standard Battery of Genotoxicity Testing for Pharmaceuticals

- Assays extant for over 30 years, essentially unchanged!
- Not changed because they serve us so well?
- Not changed because genetic toxicologists are Luddites?
- Not changed because of lack of new technologies?
“Shovel ready” new technologies

- In silico modeling
- Comet assay
- Flow cytometry for in vivo micronuclei
- Flow cytometry for in vitro micronuclei
- Pig-a gene mutation assay
- Gene expression arrays
Long term or specialized technologies

- DNA adductome
- Enzyme DNA films
- 3D reconstructed skin models
- GreenScreen HC assay
- Yeast DEL assay
In silico modeling

- Multiple programs available:
  - MultiCASE
  - Derek for Windows
  - Vitik
  - Leadscope

- Modeling can be useful:
  - Selecting leads from large numbers of candidates
  - Assessing risk of low level contaminants, e.g., impurities in drug substances and drug products
Limitations of in silico modeling

- Not useful for novel structures
- Conflicting predictions from different programs
- Programs can be tuned for either sensitivity or specificity.
- Overall accuracy is questionable.
“Comet” assay: single cell gel electrophoresis assay

- Measures strand breaks or alkali labile lesions in DNA.
- Can be applied to cultured cells and to many cell types in vivo.
Comet Assay Overview

1. DNA damage (Chemical, UV or γ-irradiation)

2. Cells with damaged (relaxed) DNA having single-strand / double-strand breaks

3. Single cells are embedded on agarose-coated slide and lysed

4. After electrophoresis and fluorescent staining, the damaged DNA is separated from the intact DNA (the "head") and generates a comet "tail".

Living cells from culture media, blood, or tissue

From Sigma Aldrich website
Flow cytometry micronucleus assays in vivo

- Endpoint not new, methods to quantify are new.
- Automation not only saves time and resources, provides more accurate assessments. Very large numbers of cells can be assessed.
Biomarkers in flow MN Assay

Two Compartments
- Bone marrow
- Peripheral blood

Two RET Markers
- RNA
- CD71

Slide from Jing Shi, BioReliance Corp
Flow Cytometric Analysis

A representative bivariates of flow cytometric analysis of MN-RETs in peripheral blood (Left: vehicle control; right: positive control).

Slide from Jing Shi, BioReliance Corp
Validation Data in Rats: 3-day Repeat Dosing

Bone Marrow

Blood

Bone Marrow

Blood

Slide from Jing Shi, BioReliance Corp
Flow cytometry for in vitro micronucleus assay

- Flow cytometry is also amendable for in vitro micronucleus analysis
- Particularly useful as an early screening assay
Flow-based *In Vitro* MN Assay

- Cells are exposed for 4 hours +S9, 24 hours –S9
- No Cytochalasin-B treatment
- A built-in cytotoxicity measurement – beads: nuclei ratio
- At lease 3 doses analyzed with top does reaching ~50% in cytotoxicity
- 2X 10000 mononucleated cells analyzed per dose
- Positive controls included

Slide from Jing Shi, BioReliance Corp
Flow vs. Manual Scoring

*, p<0.05 %MN values were analyzed by Fisher’s Exact test.

Slide from Jing Shi, BioReliance Corp

Shi et al., Mutagenesis, 25:33-40, 2010
Pig-a locus mutation assay

- The product of this gene has a critical role in the production of glycosylphophatidylinositol (GPI) anchors, a structure used to target specific proteins to the cell surface. Disruption of this gene eliminates the expression of these proteins on the cell surface, and this phenotype can be readily identified by flow cytometric analysis.

- The *Pig-a* gene is located on the X-chromosome and thus has only one functional copy in both males and females. Therefore, as opposed to autosomal genes with two alleles, only a single mutation event or “hit” is required to affect the *Pig-a* gene’s function and produce the characteristic phenotype.

From Litron website
GPI-anchors and Pig-A

Slide from Jing Shi, BioReliance Corp
Representative Plots

Vehicle

ENU 40mg/kg/day

Slide from Jing Shi, BioReliance Corp
Acute Dosing Data (3 doses)

Slide from Jing Shi, BioReliance Corp
Toxicogenomic analysis

- In vitro mammalian genetox assays (cytogenetics, mouse lymphoma) generate a high rate of positive results, 25-30%.
- Many of these are later found to be false positives because they are not carcinogens and don’t demonstrate genotoxic responses in vivo.
- Gene expression analyses can be used to distinguish true genotoxic positives from false positives.
Visualization of Genotoxic Biomarker

Heatmap of 58 Gene Genomic Biomarker

Genotoxic Induced

Genotoxic Repressed

Li, Hyduke, Aubrecht and Fornace
Caffeine - hypothetical drug candidate

Mechanism of action:
- CNS stimulatory effects occur via competitive antagonism at adenosine receptors

Indication:
- CNS, ADD, CNS stimulatory

Target population:
- Widely used including women with child bearing potential

Exposure:
- Chronic 200-300mg daily

Genetox profile:
- Ames – negative
- **In vitro chrom abs – positive**
- In vivo Chrom abs – negative

• Key questions:
  - Relevance of positive findings?
  - DNA reactive vs. non-reactive mechanisms?

From Jiri Aubrecht, 2008
Caffeine Does Not Activate Genotoxic Stress Response

Genomic biomarker of genotoxic stress response

- DNA damaging
- Non-damaging
Proposed new recommended test battery
(as at ICH EWG Brussels meeting May 2007)

A test for gene mutation in bacteria

Option 1
- In vitro mammalian cell test
  - Negative
  - Integrated MNT
    - no 2nd end-point/tissue
  - Positive
  - MNT
    - plus 2nd end-point/tissue

Option 2
- No in vitro mammalian cell test
  - Only impact is on drug label
  - MNT
    - plus 2nd end-point/tissue