S2(R1) — Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use —

Speaker	Makoto HAYASHI, D.Sc.
Title	Director General
Organisation	BioSafety Research Center

Disclaimer:

The information within this presentation is based on the presenter's expertise and experience, and represents the views of the presenter for the purposes of a training workshop.

Background

The recommendations from the latest Organization for Economic Co-operation and Development (OECD) guidelines and the reports from the International Workshops on genotoxicity Testing (IWGT) have been considered where relevant. In certain cases, there are differences from the OECD or IWGT recommendations, which are noted in the text. The following notes for guidance should be applied in conjunction with other ICH guidances.

Background

- Too many positives in the *in vitro* mammalian cells assay systems that may not be relevant to human risk
- Newer developments, such as *in vitro* micronucleus, rat blood micronucleus, newer experience with *in vivo* testing such as Comet
- Taking into consideration of 3R's for genotoxicity assays whenever possible "without impacting" the scientific value of the tests and the evaluation of the human risk.

Objective of the Guideline

This guidance replaces and combines the ICH S2A and S2B guidelines. The purpose of the revision is to optimize the standard genetic toxicology battery for prediction of potential human risks, and to provide guidance on interpretation of results, with the ultimate goal of improving risk characterization for carcinogenic effects that have their basis in changes in the genetic material.

Scope of the Guideline

The focus of this guidance is testing of new "small molecule" drug substances, and the guidance does not apply to biologics. Advice on the timing of the studies relative to clinical development is provided in the ICH M3 (R2) guidance.

Summary of major points of the revisions

- **S2A and S2B guidances merged into one**
- **Options provided for the test battery**
  - Battery with *in vitro* mammalian cell assay
  - Battery without *in vitro* mammalian cell assay but two *in vivo* assays on two tissues

Two Options for the Standard Battery (1)

- **Option 1**
  i. A test for gene mutation in bacteria.
  ii. A cytogenetic test for chromosomal damage (the *in vitro* metaphase chromosome aberration test or *in vitro* micronucleus test), or an *in vitro* mouse lymphoma Tk gene mutation assay.
  iii. An *in vivo* test for genotoxicity, generally a test for chromosomal damage using rodent hematopoietic cells, either for micronuclei or for chromosomal aberrations in metaphase cells.

Two Options for the Standard Battery (2)

- **Option 2**
  i. A test for gene mutation in bacteria.
  ii. An in vivo assessment of genotoxicity with two different tissues, usually an assay for micronuclei using rodent hematopoietic cells and a second in vivo assay. Typically this would be a DNA strand breakage assay in liver, unless otherwise justified.

Summary of major points of the revisions

- **In vitro mammalian cell assay**
  - Reduction in top concentration from 10 to 1 mM
    - 1 mM or 0.5 mg/ml, whichever is lower
  - Tightened acceptable cytotoxicity limits
  - No longer require testing of precipitating concentrations
  - The in vitro MN is accepted as a third alternative in the in vitro assays

Summary of major points of the revisions

- ***In vivo* cytogenetic assay**
  - Rat blood is now acceptable for *in vivo* MN analysis

Cytotoxicity in *in vitro* mammalian assays

- ***In vitro* metaphase chromosome aberrations or micronuclei**
  - it is not necessary to exceed a reduction of about 50% in cell growth
- **Mouse lymphoma *tk* mutation assay**
  - reduction of about 80% in RTG (relative total growth)

Summary of major points of the revisions

- ***In vitro* bacterial mutation assay no longer requires duplicate assay**
- **Integration of genotoxicity endpoints into routine toxicology studies**
  - stringent criteria defined for acceptability of top dose (option 2 and follow up +ve in option 1)
- **Combination two *in vivo* assays by acute exposure, *e.g.*, micronucleus + comet assays**

Criteria for acceptable dose/exposure in (sub)chronic study (1)

- **Maximum feasible dose (MFD) based on physico chemical properties of the drug in the vehicle**
- **Limit dose of 1000 mg/kg for studies of 14 days or longer, if this is tolerated**
- **Maximal possible exposure**
  - plateau/saturation in exposure
  - accumulation of the compound

Criteria for acceptable dose/exposure in (sub)chronic study (2)

- **Substantial reduction in exposure to parent drug with time (e.g., ≥ 50% reduction from initial exposure) should not be used as top dose**
- **Top dose is ≥50% of the top dose that would be used for acute administration if such acute data are available for other reasons**

Summary of major points of the revisions

- **Advice on choice of second *in vivo* genotoxicity endpoint**
  - includes Comet assay and also DNA strand break assay, decreases emphasis on UDS assay
- **Provided advice on weight of evidence and data evaluation to determine relevance of positive findings**

Assessment of biological relevance

- **Small increases in apparent genotoxicity *in vitro* or *in vivo* should first be assessed for reproducibility and biological significance. Examples of not considered biologically meaningful:**
  - Small increases that are statistically significant compared with the negative or solvent control values but are within the confidence intervals of the appropriate historical control values for the testing facility.
  - Weak/equivocal responses that are not reproducible.

Benefits of revisions: The 3 R's (1)

- **No longer require concurrent positive controls in every *in vivo* assay**
- **Integration of genotoxicity into toxicology assays or the combination assay with multiple endpoints**
- **Reduction in "non-relevant" *in vitro* results will reduce number of follow-up *in vivo* assays**

Benefits of revisions (2)

- Incorporates accumulated knowledge specific to testing of pharmaceuticals
- Takes advantage of new technologies
- More options in the test battery

Benefits of revisions (3)

- **Reduction in delays caused by dealing with "non-relevant" *in vitro* positive genotoxicity results**
- **More efficient use of resources**

Conclusion

- S2A and S2B guidances merged into one
- Options provided for the test battery
- Reduction of irrelevant positives in *in vitro* mammalian cell assay
- *In vivo* assays can be incorporated into repeat dose toxicological assays
- No longer require concurrent positive controls in every *in vivo* assay
- *In vitro* bacterial mutation assay no longer requires duplicate assay

ICH
harmonisation for better health

Thank You!