The 2nd JaCVAM Workshop
April 17, 2009
NIHS

コメットアッセイの国際バリデーション研究

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遺伝毒性試験

	DNA 傷害性	遺伝子突然変異	染色体異常 誘発性
in vitro	*rec*アッセイ, コメット試験, UDS試験	Ames試験 MLA	CHL, ヒト末梢血, MLA
in vivo	コメット試験, UDS試験	トランスジェニック動物試験	げっ歯類を用いる小核試験

Available online at www.sciencedirect.com
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Mutation Research 584 (2005) 1–256
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity☆

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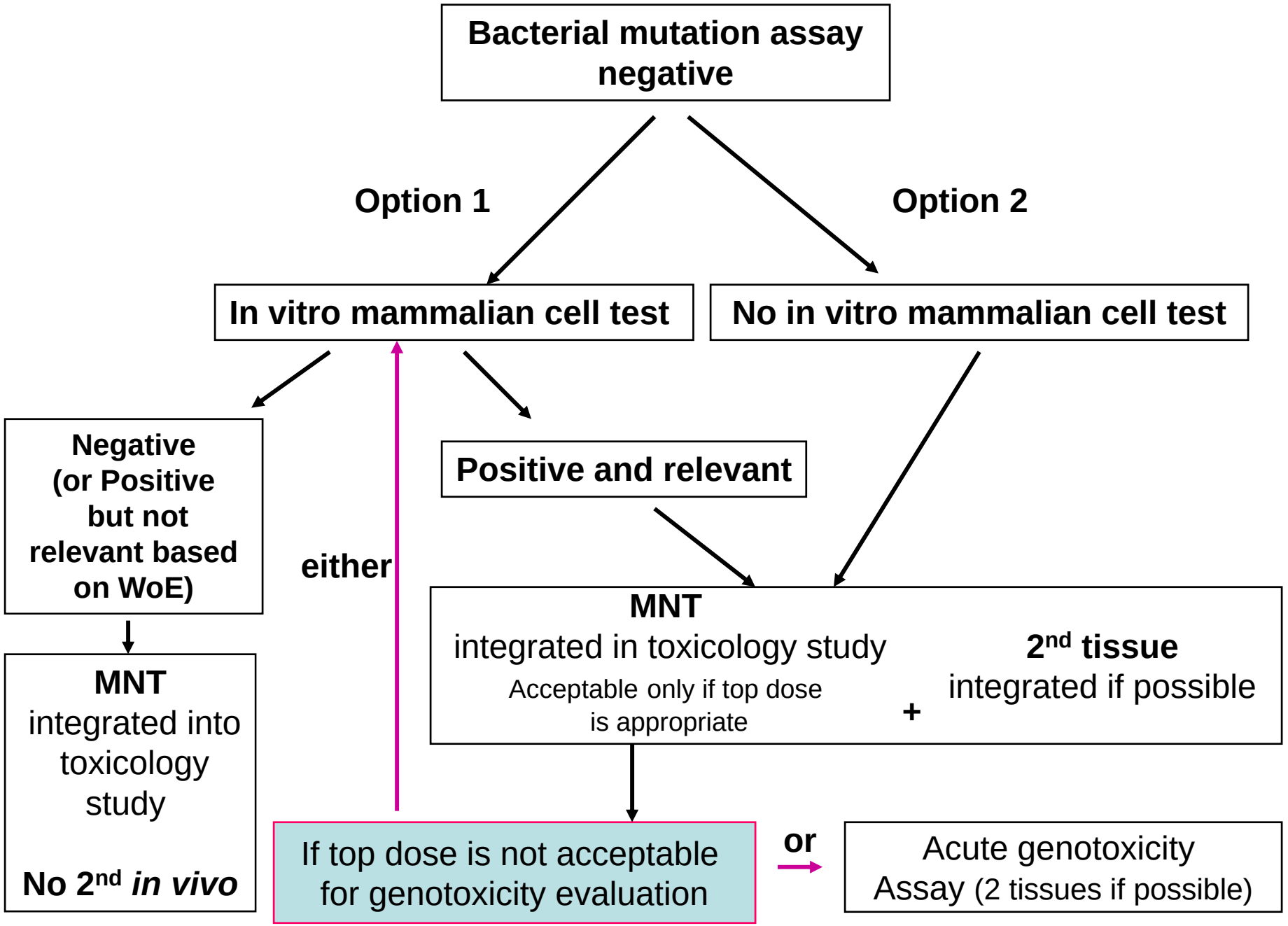
Motivations of revision

- ***Too many positives in the in vitro mammalian cells assay systems that may not be relevant to human risk***
- ***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.***

Summary of major points of the revisions

- S2A and S2B guidances merged into one
- Options provided for the test battery
  - O-1 Battery **with** *in vitro* mammalian cell assay
  - O-2 Battery **without** *in vitro* mammalian cell assay but two *in vivo* assays
- *In vitro* mammalian cell assay
  - Reduction in top concentration **from 10 mM to 1 mM**
  - Tightened acceptable cytotoxicity limits
  - No longer require testing of precipitating concentrations
- *In vitro* bacterial mutation assay no longer requires duplicate assay


Bacterial mutation assay negative
Option 1
Option 2
In vitro mammalian cell test
No in vitro mammalian cell test
Negative (or Positive but not relevant based on WoE)
Positive and relevant
either
MNT integrated in toxicology study
Acceptable only if top dose is appropriate
+
2nd tissue integrated if possible
MNT integrated into toxicology study
No 2nd in vivo
If top dose is not acceptable for genotoxicity evaluation
or
Acute genotoxicity Assay (2 tissues if possible)

Benefits of revisions: The 3 R's

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

ECVAM Workshop

Reduction in Regulatory Genotoxicity Testing: Identification and Implementation Opportunities

Ranco (VA), Italy

24th – 25th June, 2008

Combination and integration in repeat dose tox studies

- Ideally Comet and MN would be integrated into repeated dose tox
- Lack of experience
- Most uncertainties are related to Comet assay
- Dosing 3h before sacrifice for Comet is needed, but may influence the general tox parameters or organ toxicity (false positives)

Combination and integration in repeat dose tox studies

- Addition of positive control group needed?
- Practical/logistic problems?
- Can increased proliferation as results of repeat dosing lead to false positive results in Comet assay? Histopathological samples would be helpful for interpretation of Comet data
- If you have accumulation of compound in tissues it would be preferred to integrate Comet and MN in repeat dose study (instead of acute/stand alone)

Summary

- Integration of MN is highly recommended
- Combination of MN and Comet in one acute test is recommended
- Integration of Comet in repeat dose tox test is not yet mature enough, more data/experience are needed

Feb. 4, 2009
Comet mtg.

In Vivo Comet Assay: Update on the On-Going Validation Coordinated by JaCVAM

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Introduction

An *in vivo* rodent alkaline Comet assay is practically used worldwide for detecting genotoxic chemicals, and it is expected as a second *in vivo* genotoxicity test in the revised ICH-S2 guidance.

The assay, however, has not been validated formally, and the international validation study is now on-going coordinated by JaCVAM. The purposes are to validate the *in vivo* Comet assay as a potential predictor of carcinogens and as an alternative follow-up assay to the more commonly used *in vivo* rodent UDS assay.

Organization Update (in vivo)

Validation Management Team (VMT)

M. Hayashi (Chair, BSRC)
R. Corvi (ECVAM)
M. Honma (NIHS)
L. Schechtman (Consultant)
R. Tice (NTP/ICCVAM)
Y. Uno (MTPC, JEMS/MMS)

Secretariat
H. Kojima (NIHS/JaCVAM)

Leading Laboratory Team (LLT)

BioReliance (B. Krsmanovic, et al.)
FDSC (K. Yamakage, et al.)
HLS (B. Burlinson, et al.)
Merck (R.D. Storer, et al.)

Consultation Team (CT)

N. Asano (JEMS/MMS)
P. Escobar (Boehringer-Ingelheim)
D. Lovell (Univ. of Surrey)
T. Morita (NIHS)
M. Nakajima (BSRC)
Y. Ohno (NIHS/JaCVAM)
T. Omori (Kyoto Univ.)
YF. Sasaki (Hachinohe Nat.Coll.Tech.)

Local Committee in JPN

Mainly from JEMS/MMS members

Progress of Validation Effort (in vivo)

2006

Aug.: Kick-off mtg. and Start of 1st validation study with EMS
Dec.: End of exp. of 1st validation study
- **Study protocol was optimized overall**
- **Well-validated data were obtained**

2007

Apr.: Start of 2nd validation study with EMS and three coded chem.
Jul.: Announcement of invitation to participate in this validation effort

2008

Jan.: Start of exp. to select participants of 4th (definitive) validation study
Mar.: End of exp. of 2nd validation study
- **Data acceptance criteria were set based on negative & positive cont. data**
- **Especially, 2,4- & 2,6-DAT showed a little bit complicated results**

May: 3rd validation study with EMS and additional 3 coded chem.
Aug.: Participants of 4th validation study were selected
Dec.: End of exp. of 3rd validation study

2nd Phase Validation Study

- **Purpose: by using data of negative control and EMS,**
  - ✓ **To determine data acceptance criteria**
  - ✓ **To examine within/between lab variability**
- **Test compound: EMS* and Three coded chemicals****
  - *** Each exp. for coded chemicals included EMS group as a positive control, and 3 data of EMS/lab X 5 labs = 15 data were applied to determine data acceptance criteria**
  - **** Acrylamide, 2,4-diaminotoluene, 2,6-diaminotoluene**
- **Protocol: version 12**
- **Result:**
  - ✓ **Data acceptance criteria (draft) were set**
  - ✓ **Data of three coded chemicals were obtained**

Data Acceptance Criteria (draft)*
based on 2nd phase validation study results

a. Negative control

- **Mean of %DNA in tail in liver: 1-8%**
- **Mean of %DNA in tail in stomach: 1-30% (preferably 1-20%)**

b. Positive control: EMS, 200 mg/kg, single (or twice) p.o.

- **Effect (ratio of means of %DNA in tail between EMS & vehicle) in liver and stomach: 2-fold or higher**
- **Effect (difference of means of %DNA in tail between EMS & vehicle) in liver and stomach: 5% or higher**
- **CV of Effect (ratio) in two or more independent experiments with liver and stomach: 50% or less**

* Data acceptance criteria may be revised based on the 3rd phase validation results

Summary

- ✓ **Expects: Acrylamide is clearly but not so strongly positive in both/either organs. 2,4-DAT and 2,6-DAT are weakly positive and negative in liver, respectively (unknown in stomach).**
- ✓ **Overall results: acrylamide was positive in both organs (one laboratory seemed not to detect this chemical as positive).**
- ✓ **In liver, 2,4-DAT was positive in labs. #2 and 3. 2,6-DAT was negative except for lab. #3. 2,6-DAT results may almost fit the expected assay results, but 2,4-DAT results may be a little bit unexpected. Overall, this validation study results may be coincident with the rat liver UDS assay results, because 2,4- and 2,6-DAT are reported as weakly positive and negative in the UDS assays, respectively.**
- ✓ **In stomach, 2,4- and 2,6-DAT seem positive in lab. #2 and 3. Both chemicals are mutagens and may have genotoxic potential *in vivo*, and *in vivo* Comet assay may sometimes detect such weakly genotoxic effects.**

Facilities and Participants of 4th Phase Validation Study

1. **AstraZeneca (UK) : Catherine Smith**
2. **Bayer HelthCare (Germany) : Uta Wirnitzer**
3. **BioReliance (USA) : Buba Krsmanovic**
4. **Covance (UK) : Lucinda Williams**
5. **Food and Drug Safety Center (JPN) : Kohji Yamakage**
6. **Health Canada (Canada) : James P. McNamee**
7. **Huntingdon Life Sciences (UK) : Brian Burlinson**
8. **Johnson & Johnson (Belgium) : Marlies De Boeck**
9. **Merck (USA) : Richard D. Storer**
10. **Mitsubishi Chemical Safety Institute (JPN) : Kazunori Narumi**
11. **Novartis Pharma (Switzerland) : Ulla Plappert-Helbig**
12. **Sumitomo Chemical (JPN) : Sachiko Kitamoto**
13. **The Institute of Environmental Toxicology (JPN) : Kunio Wada**

Outlines of On-going/Next Phase Validation Studies

- **Study and purpose:**
  a. **3rd phase validation study: ongoing**
     To reconfirm data acceptance criteria based on 2nd phase validation data, and To further optimize the standard protocol
  b. **4th phase validation study: now planning**
     To investigate predictive capacity of genotoxic carcinogens
- **Test compound:**
  a. **Coded three chemicals plus EMS in 3rd phase validation**
  b. **Coded "the number of 30-50" chemicals in 4th phase validation**
- **Participant:**
  a. **4 leading lab for 3rd phase validation**
  b. **4 leading lab plus selected lab (max. 9) for 4th phase validation**
- **Method: In accordance with the standard protocol**
- **Schedule:**
  a. **March/2008 – February/2009 in 3rd phase validation**
  b. **Start on 1Q/2009 for 4th phase validation**
     Finish by the end of 2010 (tentative)

EFPIA/PHRMA initiative on integration of genotoxicity assays into general toxicity studies