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JaCVAM
1st International Workshop
Tokyo March 11, 2008
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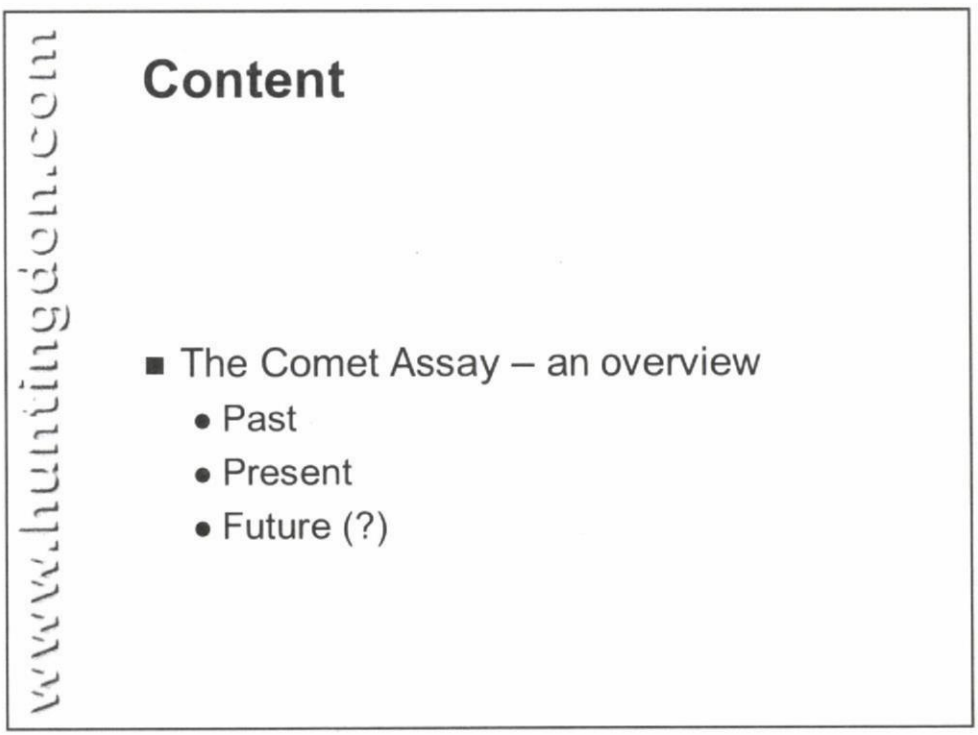
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Content
■ The Comet Assay – an overview
• Past
• Present
• Future (?)

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## Is there a place for the Comet assay in the current test battery?

- Battery is there to identify hazard
- In vitro – do we need another assay?
  - General consensus was probably not
- In vivo – do we need another assay
  - General consensus is yes we do

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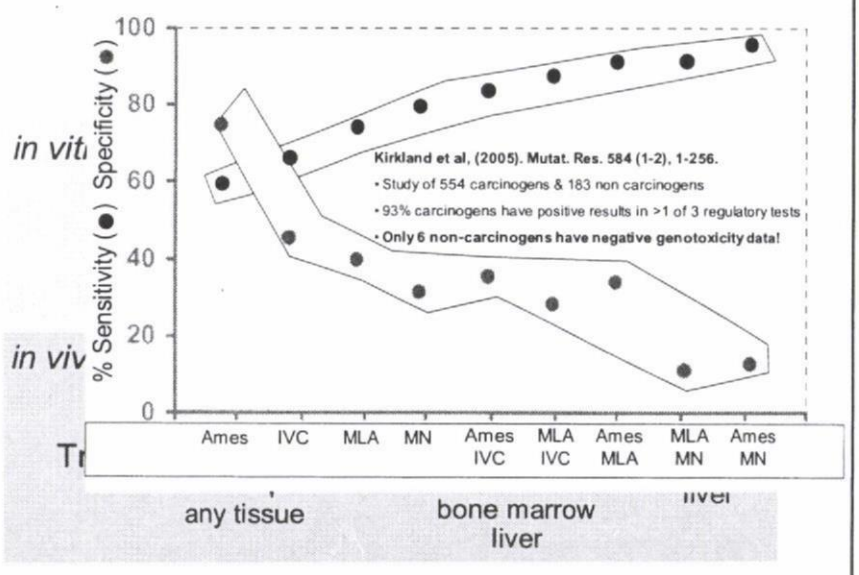

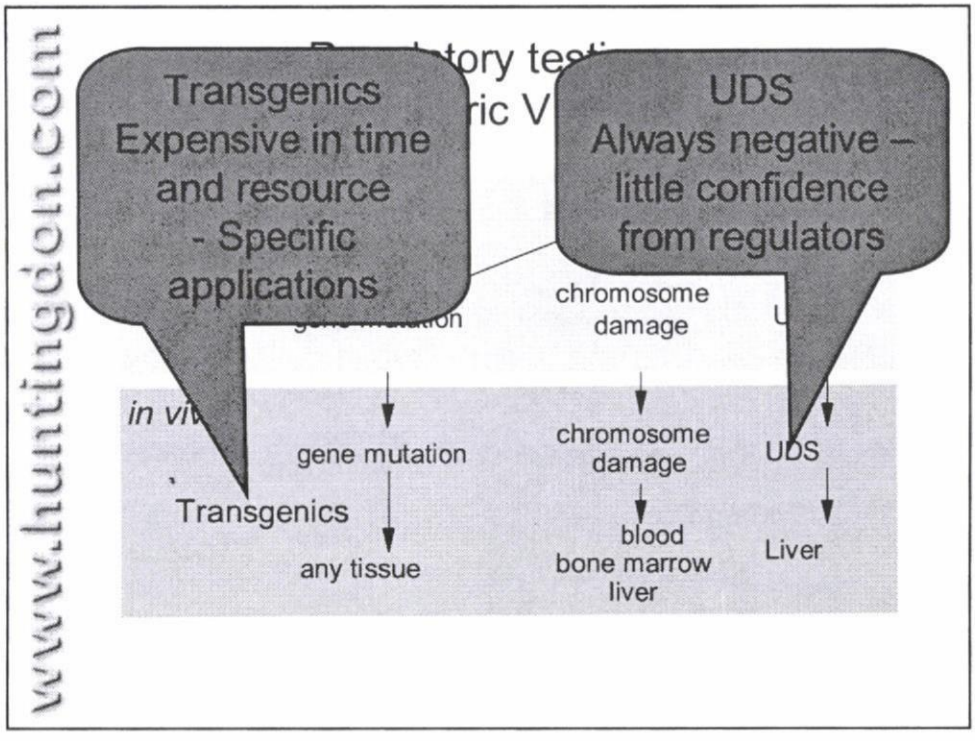
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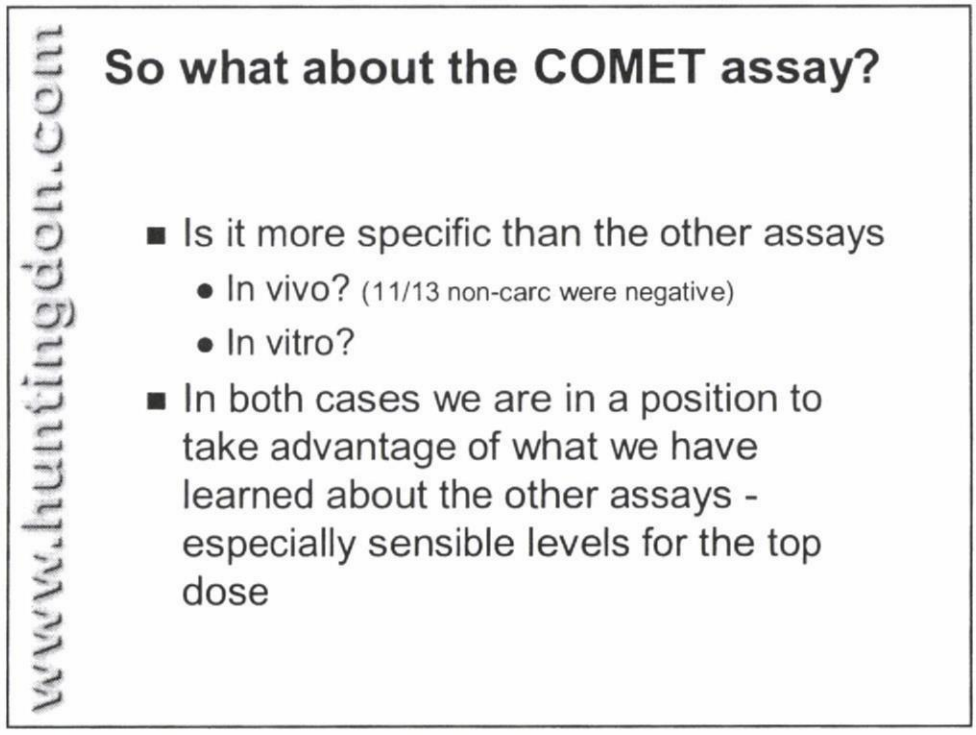
Transgenics
Expensive in time and resource - Specific applications
UDS
Always negative – little confidence from regulators
chromosome damage
gene mutation
chromosome damage
UDS
Transgenics
any tissue
blood
bone marrow
liver
Liver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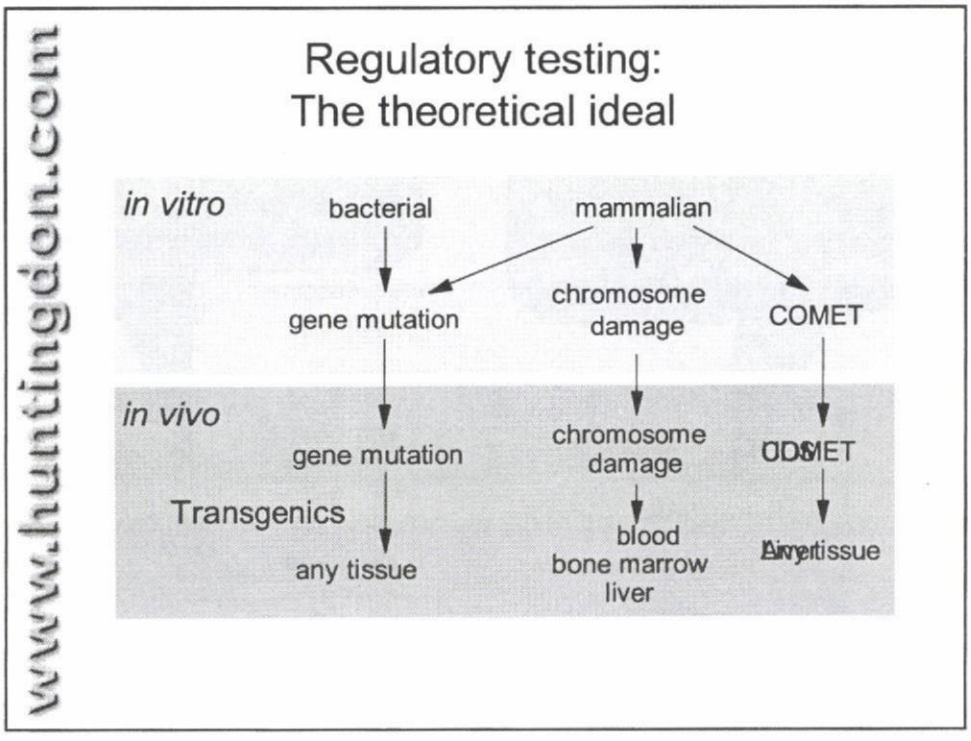
So what about the COMET assay?
Is it more specific than the other assays
In vivo? (11/13 non-carc were negative)
In vitro?
In both cases we are in a position to take advantage of what we have learned about the other assays - especially sensible levels for the top dose




| Drug candidates with genotoxicity data sets triggering comet assay in vivo | | | | |
|---|---|---|---|---|
| Drug | Available Results | Tissue(s) Comet assay | Comet Assay | Consequence for compound |
| A | Ames -<br>MNT V79 -<br>CAT V79 +<br>CAT HuLy -<br>MNT BM rat - | **Liver, leukocytes (oral 3 and 24-hour sampling)** | **negative** | **ok from HA / EC for multiple dose clinical studies in patients** |
| B | Ames -<br>MNT V79 -<br>Comet HuLy -<br>CAT V79 +<br>MNT BM rat - | **Liver (oral; 3 and 24-hour sampling)** | **negative** | **ok from HA/ EC for first administration to humans** |
| C | Ames +/-<br>MNT V79 +<br>CAT V79 +<br>CAT HuLy +/-<br>MLA TK +<br>HPRT CHO -<br>Comet V79 +<br>MNT BM mouse -<br>UDS liver rat - | **Liver, leukocytes (sub-cut; 3 and 24-hour sampling)** | **negative** | **ok from HA / EC for first (topical) administration to humans** |

| Examples of drug candidates with tumour findings triggering comet assay in vivo | | | | | |
|---|---|---|---|---|---|
| | **Available results** | ·Tumor target organ | ·Tissue and time Comet assay | ·Result of Comet Assay | ·Conclusion |
| ·D | Ames -<br>MNT V79 -<br>CAT V79 -<br>HPRT V79 -<br>MNT BM mouse -<br>UDS liver rat - | Small intestine<br>Rat | Jejunum, Liver<br>(oral admin.)<br>3 and 24 hour sampling | negative | No unacceptable health risk<br>(non-genotoxic mechanism, epigenetic mechanism) |
| ·E | Ames -<br>MNT V79 -<br>Comet V79 -<br>CAT CHO -<br>HPRT V79 -<br>HPRT CHO -<br>MNT BM rat - | Liver<br>Rat | Liver<br>(oral 3 and 24 hour sampling | Negative | No unacceptable health risk<br>(non-genotoxic mechanism, epigenetic mechanism) |

| Examples of drug candidates with data of comet assay in vivo using tissue of site of first contact | | | | |
|---|---|---|---|---|
| Drug | Available results | Tissue(s) investigated | Result | Outcome |
| ·F | Ames +/-<br>MNT V79 (+)<br>CAT HuLy -<br>MNT BM mouse -<br>DNA binding rat -<br>MutaMouse - | Jejunum, liver<br>(oral;3 and 24-hour sampling) | negative | ok from health authorities to proceed into multiple dose clinical trials |
| ·G | Ames -<br>MNT V79 +<br>Comet V79 +<br>CAT HuLy +<br>MNT BM rat - | Stomach mucosa<br>(oral; sampling time 3 hours) | Retarded DNA migration (reason: DNA-protein crosslinks) | Termination of development |

## Other Comments

- Lack of agreed criteria for collection, presentation and analysis of results
- Uncertainty about toxicity
- Large volume of published data – lots of false "positives"

- But assay accepted in EU

Andrew Smith (UK HSE)



- Large number of procedures (protocols)
- Variance in presenting results
- Little regulatory experience in evaluation.
- Common for "new" assays

Jon Battershill (DoH UK)

- Has important potential but many technical shortcomings
- Considered a tier 2 test for following up ICH battery or
- To exclude genotoxic MOA in positive carcinogenicity studies

David Jacobson-Kram FDA



## JaCVAM trial

- It is clear that a lot of the concerns could be removed once there is an accepted OECD guideline
- The JaCVAM trial is aiming towards that goal
- International committee should ensure fast track through to acceptance

## 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 endpoints
- 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



## Summary of major points of the revisions, continued

- Integration of genotoxicity endpoints into routine toxicology studies
  - Stringent criteria defined for acceptability of top dose
- Advice on choice of second in vivo genotoxicity endpoint
  - includes Comet assay, decreases emphasis on UDS assay)
- Provided advice on weight of evidence and data evaluation to determine relevance of positive findings

## Timing of genotoxicity studies for Phase 1

- Option 1; two vitro tests only
  - in the past this led to many in vivo assays before FIM, to follow up positive in vitro results
- Option 2: one in vitro test (Ames) and two in vivo endpoints, preferably integrated into toxicology study



## 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

## Benefits of revisions:

- Incorporates accumulated knowledge specific to testing of pharmaceuticals
- Takes advantage of new technologies
- More options in the test battery
- Reduction in delays caused by dealing with "non-relevant" in vitro positive genotoxicity results
- More efficient use of resources



## Expected targets

- Finalization of text of draft guideline
- Ensure ultimate acceptance by groups represented by EWG
- Postal sign off for step 2
  - end December 2007
- Publication in the regions and regional consultation period
- Step 4 in June 2008 in Portland pending speed of Federal Register publication and comment period

# HLS JaCVAM Data

- 3 unknown compounds supplied by JaCVAM panel



# Tissues to date!

| Tissue Type | Number of Males | | Number of Females | | Total Number | |
|---|---|---|---|---|---|---|
| | Vehicle | Positive | Vehicle | Positive | Vehicle | Positive |
| Bone Marrow | 18 | 15 | 13 | 11 | 31 | 26 |
| Liver | 46 | 48 | 14 | 12 | 60 | 60 |
| Stomach | 40 | 41 | 5 | 4 | 45 | 45 |
| Peripheral Blood | 22 | 21 | 3 | 3 | 25 | 24 |
| Kidney | 23 | 19 | 5 | 5 | 28 | 24 |
| Duodenum | 17 | 13 | 5 | 5 | 22 | 18 |
| Colon | 18 | 13 | 5 | 4 | 23 | 17 |
| Ileum | 15 | 14 | 4 | 4 | 19 | 18 |
| Jejunum | 9 | 8 | 3 | 3 | 12 | 11 |
| Testes | 13 | 11 | | | 13 | 11 |
| Lung | 4 | 3 | 3 | 3 | 7 | 6 |
| Bladder | 3 | 1 | 1 | 1 | 4 | 2 |
| Mammary Gland | | | 2 | 2 | 2 | 2 |
| Ovaries | | | 1 | 1 | 1 | 1 |
| | | | Number of Tissues | | 292 | 265 |
| | | | Total Number of Tissues | | 557 | |

## Liver

| Compound 7 | Original Results | Repeated Results |
|---|---|---|
| **(%) Tail intensity fold increase over vehicle:** | 5 | 3 |
| **Tail Moment fold increase over vehicle:** | 7 | 3 |
| **Tail Length fold increase over vehicle:** | 2 | 1 |
| **Compound 8** | | |
| **(%) Tail intensity fold increase over vehicle:** | 4 | 3 |
| **Tail Moment fold increase over vehicle:** | 5 | 3 |
| **Tail Length fold increase over vehicle:** | 2 | 2 |
| **Compound 9** | | |
| **(%) Tail intensity fold increase over vehicle:** | 3 | 3 |
| **Tail Moment fold increase over vehicle:** | 5 | 3 |
| **Tail Length fold increase over vehicle:** | 2 | 1 |



## Stomach

| Compound 7 | Original Results | Repeated Results |
|---|---|---|
| (%) Tail intensity fold increase over vehicle: | 3 | 3 |
| Tail Moment fold increase over vehicle: | 3 | 3 |
| Tail Length fold increase over vehicle: | 2 | 1 |
| **Compound 8** | | |
| (%) Tail intensity fold increase over vehicle: | 2 | 2 |
| Tail Moment fold increase over vehicle: | 2 | 3 |
| Tail Length fold increase over vehicle: | 2 | 2 |
| **Compound 9** | | |
| (%) Tail intensity fold increase over vehicle: | 2 | 2 |
| Tail Moment fold increase over vehicle: | 2 | 2 |
| Tail Length fold increase over vehicle: | 1 | 2 |

## Bone Marrow

**Compound 7**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 2 |
| Tail Moment fold increase over vehicle: | 2 |
| Tail Length fold increase over vehicle: | 1 |

**Compound 8**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 2 |
| Tail Moment fold increase over vehicle: | 3 |
| Tail Length fold increase over vehicle: | 2 |

**Compound 9**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 2 |
| Tail Moment fold increase over vehicle: | 2 |
| Tail Length fold increase over vehicle: | 1 |



## Kidney

**Compound 7**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 6 |
| Tail Moment fold increase over vehicle: | 9 |
| Tail Length fold increase over vehicle: | 2 |

**Compound 8**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 3 |
| Tail Moment fold increase over vehicle: | 4 |
| Tail Length fold increase over vehicle: | 1 |

**Compound 9**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 5 |
| Tail Moment fold increase over vehicle: | 8 |
| Tail Length fold increase over vehicle: | 2 |

## Colon

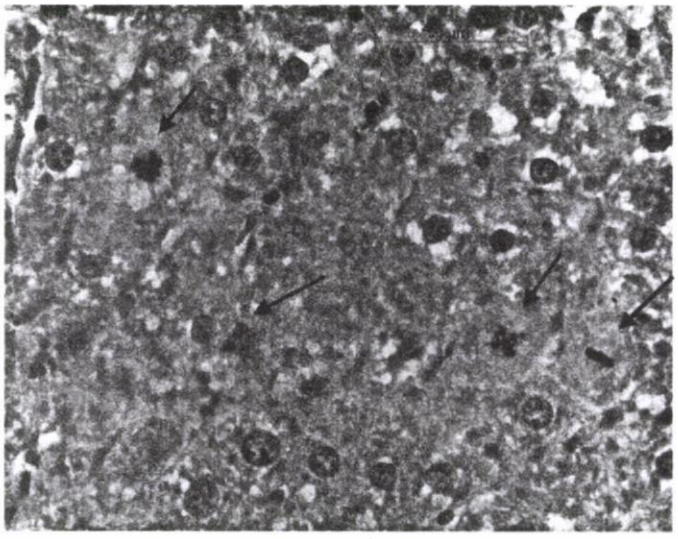
**Compound 7**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 4 |
| Tail Moment fold increase over vehicle: | 5 |
| Tail Length fold increase over vehicle: | 2 |

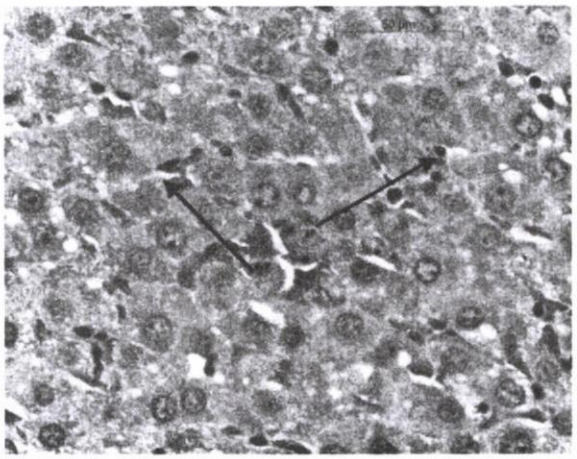
**Compound 8**

| | |
|---|---|
| (%) Tail intensity fold increase over vehicle: | 3 |
| Tail Moment fold increase over vehicle: | 3 |
| Tail Length fold increase over vehicle: | 2 |





Animal No. 233 (Compound 7) Liver
Increased numbers of mitotic figures
(mitotic figures indicated by arrows).



Animal No. 432 (Compound 9) Liver
Increased apoptosis (apoptotic cells indicated by arrows).



- Thank you and any questions?

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

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Mitsubishi Tanabe Pharma Co.
JEMS/MMS

## 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

*Validation Management Team (VMT)*
M. Hayashi (Chair, JaCVAM/NIHS)
R. Corvi (ECVAM)
M. Honma (NIHS)
L. Schechtman (Consultant)
R. Tice (NIEHS)
Y. Uno (MTPC, JEMS/MMS)

Secretariat
H. Kojima (JaCVAM/NIHS)

*Consultation Team*
N. Asano (Nitto Denko, JEMS/MMS)
D. Lovell (Univ. of Surrey)
T. Morita (NIHS)
N. Nakashima (PMDA)
Y. Ohno (JaCVAM/NIHS)
T. Omori (Kyoto Univ.)
YF. Sasaki (Hachinohe Nat.Coll.Tech.)

*Leading laboratory*
Anpyo-Center (M. Nakajima, et al.)
BioReliance (formerly P. Escobar, et al.)
FDSC (K. Yamakage, et al.)
HLS (B. Burlinson, et al.)
Merck (R.D. Storer, et al.)

*Local Committee in JPN*
Mainly from JEMS/MMS members

## Outline of *in vivo* Comet Assay Validation

## The 1st Phase Validation Study on *in vivo* Comet Assay – almost finished

- Purpose: Optimization of study protocol
- Test comp: Ethyl methanesulfonate (EMS)
- Solvent: Physiological saline
- Dose: 0, 100, 200 mg/kg, p.o.
- Animal: CD(SD) rat, male, 5 animals/group
- Administration: Twice (21 or 24 hours interval)
- Organ tested: Liver, Glandular stomach
- Cell preparation: 3 hours after 2nd administration, mincing for liver, scraping for stomach
- Electrophoresis: 300 mA, 15-40 min, below 10℃ (2-20℃)
- Image analysis: Use of Comet IV, SYBR Gold staining
- Cell observed: 50 cells/slide, duplicate

## The 2nd Phase Validation Study on *in vivo* Comet Assay – ongoing

- Purpose:
  - ✓ Investigate intra-/inter-laboratory validation with EMS
  - ✓ Decide acceptable variation criteria on Effect for the 3rd phase (main) validation studies
- Test comp: Three coded chemicals and EMS,
  Each exp. for coded chemicals should include an EMS group, and thus 3 data of EMS/Lab. X 5 Labs. = 15 data available, then analyze validity
- Solvent: Directed by VMT
- Dose: One of three chemicals was directed by VMT, Other two was decided in each laboratory
- Others: Same as the 1st phase validation study

## The 3rd Phase (main) Validation Study on *in vivo* Comet Assay – now planning

- **Purpose:** Investigate predictive capacity of carcinogens
- **Test comp:** Coded "the number of X" chemicals
- **Participant:** 5 leading lab + selected lab*
  * 15 lab hope to join, and selection process is ongoing
- **Method:** In accordance with our standard protocol
- **Schedule:** Begin at 3Q or 4Q/2008 (or 1Q/2009?)
  Finish by the end of 2010 (tentative)

◆ **In addition...**
**we will mix up existing data to evaluate the assay capacity**

## Steps of *in vivo* Comet Validation Studies (Based on ECVAM Validation Process)

- ✓ **Test definition**
  Protocol optimization, Training ... 1st phase validation
- ✓ **Within-lab. variability**
  3 exp. with EMS in each lab. ... 2nd phase validation
- ✓ **Transferability**
  May be assessed with data from candidate facilities because they have done exp. based on our standard protocol without additional information
- ✓ **Between-lab. variability**
  - EMS data from 5 lab. ... 1st & 2nd phase validation
  - 3 coded chem. data from 5 lab. ... 2nd phase validation
- ✓ **Predictive capacity**
  Exp. with "X" coded chem. in "Y" lab. ... 3rd phase (main) validation
- ✓ **Reproducibility**
  Will be evaluated with data from 1st & 2nd (& 3rd?) phase validation

- ✓ **Applicability domain**: to be discussed
- ✓ **Minimum performance standards**: to be discussed
- ✓ **Independent peer review**: to be discussed

Blue: almost finished, Green: ongoing. Red: now planning

## Data from Validation Study

## Data in the 1st Phase Validation Study on *in vivo* Comet Assay

- Parameter: %DNA in tail, Tail length, Olive tail moment
- Data analysis:

Three key terms for data analysis are

- ✓ Endpoint: individual values for each parameter
- ✓ Estimate: a mean or median calculated with values of Endpoint in each animal
- ✓ Effect: difference (or ratio) of an average of Estimate between a control group and a treatment group.

Purpose of validation study analysis is to investigate how large variation exists among data from testing facilities, and Effect would be a preferable yardstick (criterion) to understand the variation of parameters among testing facilities.