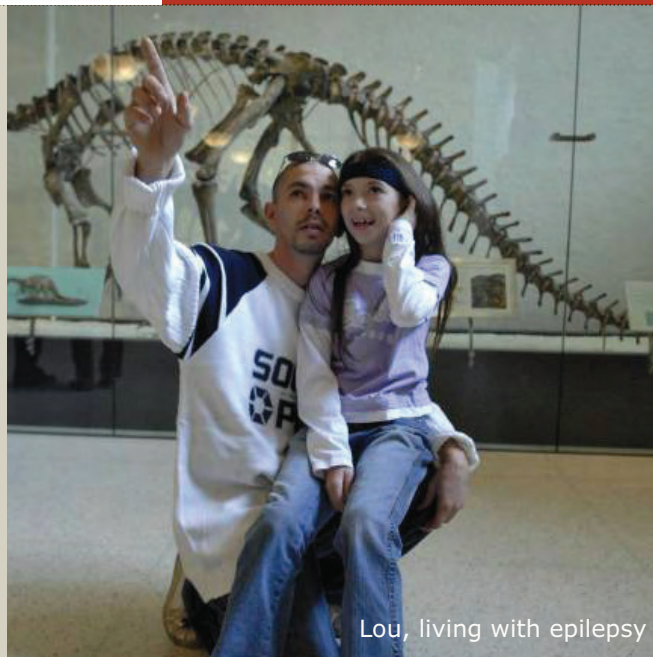


## Evaluation of a Battery of Early Genotoxicity Assays to Predict Regulatory Testing

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UCB Pharma



Lou, living with epilepsy



19/1/09

ADMET meeting, Brussels 22-23 Jan 2009

## Plan

- Screening strategy: Overview of our activities
- Genotoxicity: current problem
- Improvement of our battery
- Other relevant approaches?
- Conclusion



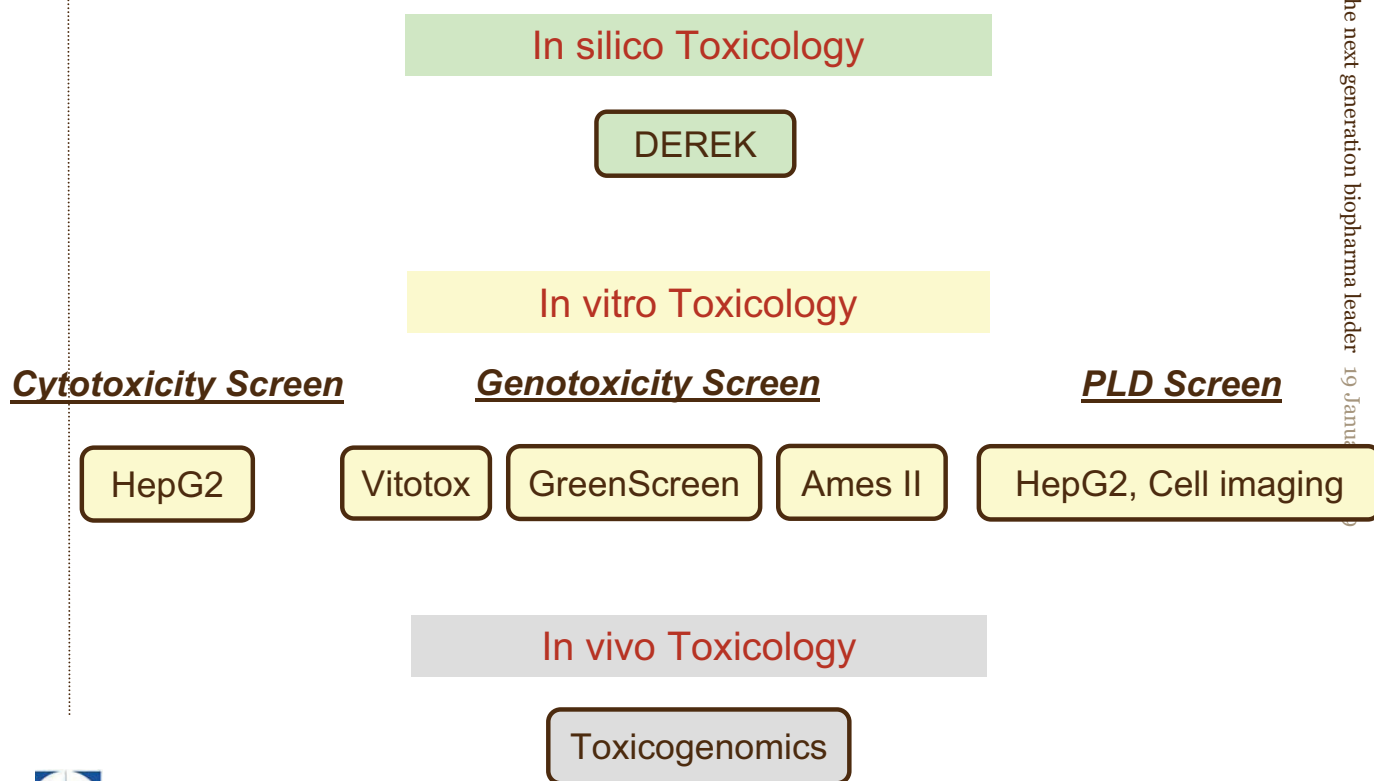
2

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# Screening strategy: overview of the activities

3

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## Genotoxicity: background information

4

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Assays required by authorities:

➤ **Before phase I clinical study:**

- **Ames test** (in vitro): detection of mutations
- **In vitro Mammalian test**: e.g mouse lymphoma assay: detection of mutations & structural chromosomal damage

➤ **Before phase II clinical study:**

- **In vivo Mammalian assay**: e.g. rodent bone marrow micronucleus assay measurement of chromosomal damage and numerical chromosomal damage

Major Drawbacks:

- Amount of cpd required
- Time consuming
- Not possible to use it for early screening

→ Need to use a battery of assays predictive of the regulatory testings



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# Genotoxicity Screening Strategy at UCB Pharma

5

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	1) In-house			2) CRO
	Vitotox (VT)	GreenScreen (GS)	Ames II (All)	Mini Ames
Amount		10 mg		250 mg
Advantages		Simple, low amount of cpd		Predictivity (Ames)
Test systems	bacteria	yeast	bacteria	bacteria
Principle:	DNA repair	DNA repair	Mutations	Mutations
Correlation:	genotox prokaryotes	genotox eukaryotes	genotox prokaryotes	genotox prokaryotes



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## Are Vitotox, GS and Ames II predictive of mini Ames?

6

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Nb of cpds (out of 35)	Vitotox	GreenScreen	Ames II	mini Ames
13	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Weak potential
5	Negative	Negative	Positive	Positive
3	Positive	Negative	Positive	Positive
2	Positive	Negative	Negative	Positive
1	Positive	Positive	Negative	Weak potential
1	Positive	Negative	Positive	Negative
1	Positive	Negative	Positive	Negative

Negative  
 Weak potential  
 Positive

### Conclusion:

- Negative cpds are correctly classified
- Cpds with medium/strong mutagenic potential are detected
- Cpds with low mutagenic potential are not detected



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## Bacterial Assay:

- **Ames MPF** (Xenometrix):
  - Same principle as the Ames II assay (detection of mutation)
  - Strains used: TA98, TA100, TA1535 and TA1537 (= Ames test)
  - NB: E. coli (uvrA et pKM101) available in 2009

## Yeast and Mammalian Assays:

- **RadarScreen** (Remynd):
  - Yeast Genetically modified yeasts (MDR pumps deleted)
  - Measurement of induction of DNA repair gene (pRAD54)
  - pRAD54 (yeast) is equivalent to GADD45a (human cells)
- **Human GreenScreen** (Gentronix):
  - Human cells (TK6) Genetically modified
  - p53 competent
  - Measurement of induction of DNA repair gene (GADD45a)



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# Objectives

- 1) Predict regulatory tests (mini Ames and micronucleus assays)
- 2) Select the best bacterial and mammalian assays
- 3) Use a bacterial and mammalian assay for screening



# Prediction of mini Ames data (mutations in bacteria)

9

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	Vitotox	Ames II	Ames MPF
SPECIFICITY	82.6	81.8	72.7
n=	23	22	22

Specificity: nb of cpds negative in Mini Ames/nb of cpds negative in assay under evaluation

	Vitotox	Ames II	Ames MPF
SENSITIVITY	18.8	37.5	68.8
n=	16	16	16

Sensitivity: nb of cpds positive in Mini Ames/nb of cpds positive in assay under evaluation



## Conclusion (prediction of mutation in bacteria)

10

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- **AMES MPF**: best assay to predict mini Ames results under evaluation
  - Prediction of weak mutagenic cpds
    - 10 cpds weakly mutagenic in mini Ames
    - 5 cpds detected in Ames MPF
    - 5 cpds not detected in Ames MPF
  - **Main differences between mini Ames assay and Ames MPF** :
    - Solid (mini Ames) versus liquid (Ames MPF) format
    - Top concentration: 5000 µg/plate or 10 mM (miniA) vs 2mM (MPF)
- NB: 3 cpds (weak positive in mini Ames, negative in Ames MPF 2mM):



Retested in Ames MPF at 10 mM: 1: +, 2: -

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