Federal Institute
for Drugs
and Medical Devices

HESI GTTC Potsdam March 22/23 2018
Applied Genetic Toxicity
for Regulatory Decision Making:
The Road Ahead

Incorporating Mode of Action into Genetic Toxicity Assessment

Dr. Roland Frötschl
Genetic and Reproductive Toxicology

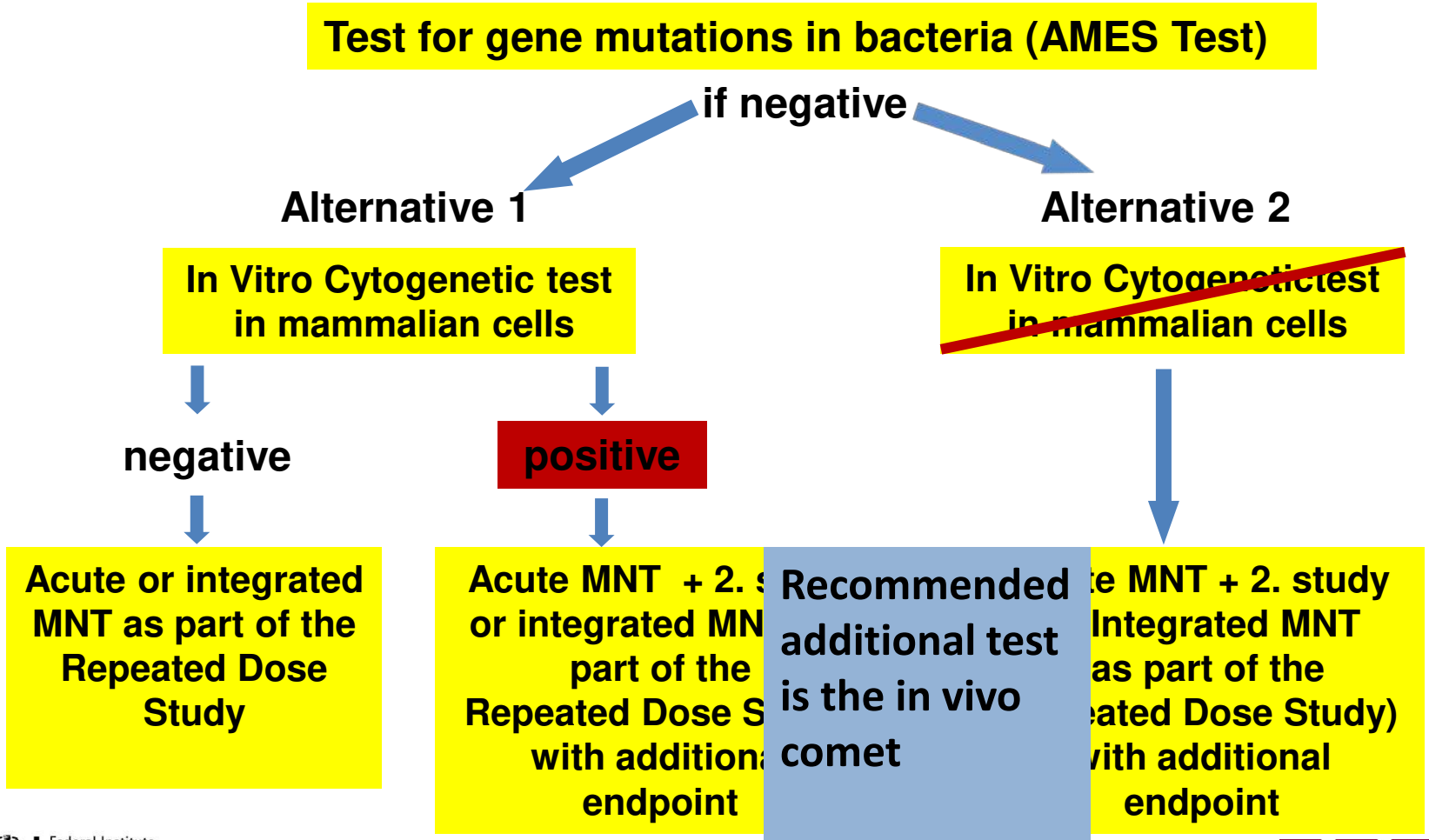
Disclaimer

Views and opinions expressed in this presentation are my own, do not necessarily reflect the opinion of other assessors at the BfArM or other EU-competent authorities, and can not be considered as BfArM or EU guidance or policy

Contents

1. Purpose of and basic test battery in genetic toxicity
2. Example for standard battery positive compound
3. Mode of action determination for risk assessment
4. Novel techniques for MoA determination to get to an AOP
5. Incorporation into risk assessment
6. Conclusions

Current Gentox Test Strategy for Pharmaceuticals CHMP/ICH/126642/08 ICH S2 (R1)



Federal Institute for Drugs and Medical Devices

Aim of the basic test strategy

- Identify mutagenic substances
  - Considered to be potentially carcinogenic
  - cause inheritable DNA-alteration which potenially lead to genetic diseases

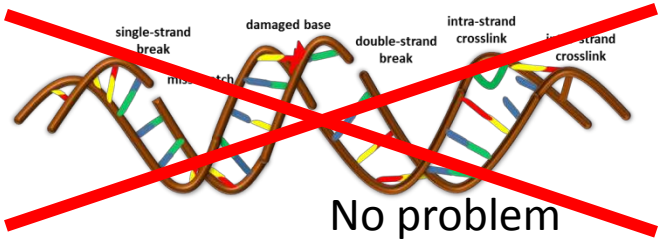
- Protect consumers/patients from any inacceptable risk for
  - developing cancer or acquiring genetic diseases
- Protect populations from any inacceptable risk for
  - Increase of genetic diseases
  - Increase in predisposition for cancer

- Standard battery is focused on the detection of inheritable DNA alterations
  - Gens mutation, chromosome mutations, genome mutations

Consequences of basic test battery results

- Fine as long as test results are negative and no hazard is identified

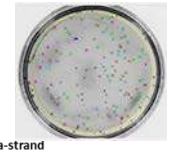
No mutation
No lesion

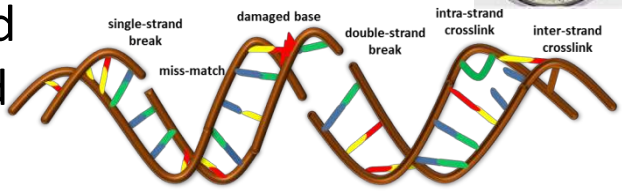


No problem

- Problems start with positive test results and the question
  - Is the hazard a risk?
  - Generally additional testing for MoA are required for the risk assessment of a genotoxic compound
  - Exclusion of direct reaction with the DNA and a dose response with a clear NOEL is required

MLA/Cab positive?!





What lesion, why,
Who???????????

Limitations of the standard battery

- with current standard genotoxicity test battery models
  - prediction of the real risk of genotoxic compounds for mutagenicity in humans is difficult or nearly impossible, guidance for MoA clarification is very limited

- What we do is to assume a worst case and get to the safe side of it

- This is very conservative and overestimates the real risk (in most cases)

Informed risk assessment improves safety assessment in genotoxicity

- This needs data on
  - Mode of action
  - Dose response and point of departure for relevant effects
  - Transspecies relevance
  - Exposure assessment
  - Environmental/human relevance

Basic quatitative risk assessment for threshold (PoD) related mechanisms

- Currently a quantitative risk assessment is only accepted for compounds
  - not directly interacting with DNA (not-DNA-reactive)

- Compounds interacting directly with DNA (e.g. alkylating agents) are still in most cases regulated conservative with a yes/no risk assessment
  - Yes means there is no PoD, any dose is considered to pose a mutagenic risk
  - Acceptability is considered only for doses with a theoretical cancer risk of 1:100,000 for pharmaceuticals or 1:1,000,000 for food additives

Determine the mode of (genotoxic)-action

- Mode of action determination needs to explain the mode of genotoxic activity
- this means practically the determination of the key activity of a molecule that leads to the final adverse outcome
- We need to know the
  - determinative molecular event (MIE molecular initiating event)
  - the dose response of the final adverse effect (gene, chromosome, genome mutations)

MoA in risk assessment
Fluoroquinolones

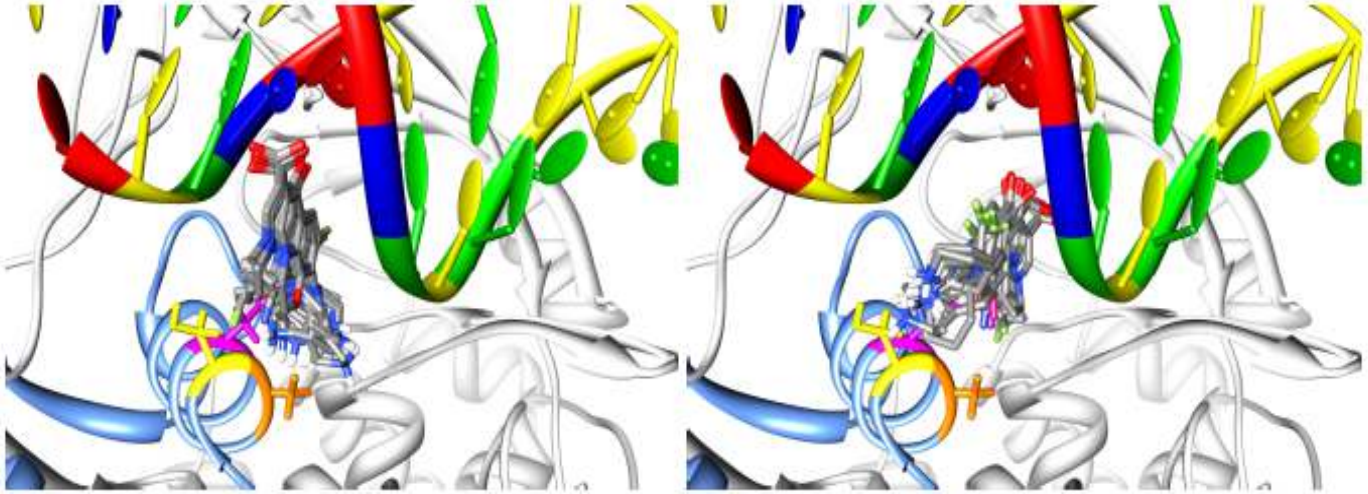


Figure 1. *Complex of DNA linked to GyrA-Tyr$_{122}$ and docked quinolones: COOH-moeities of ligands point out of (left) and into plane (right); enzyme shown in grey, QRDR in lightblue, Ser$_{83}$, Ala$_{84}$ and Asp$_{87}$ in α_4 helix in yellow, orange and magenta, resp.; G in green, C in yellow, T in blue and A in red.*

from Lenz et al. NIC Series, Vol. 40, ISBN 978-3-9810843-6-8, pp. 289-292, 2008

Mode of Action (MoA)

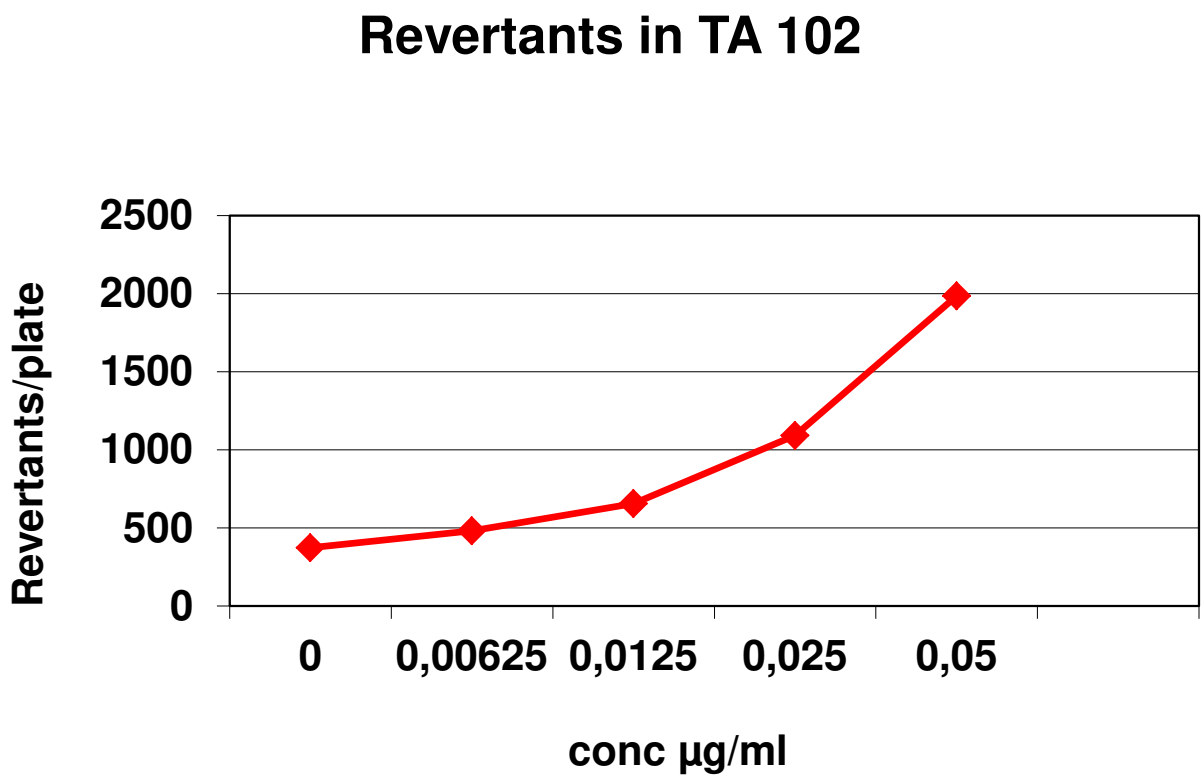
- Pharmacological (antibacterial) activity is inhibition of bacterial gyrase and topo IV inhibitor

- MoA for adverse genotoxic reactions in mammals is considered to be the inhibition of mammalian topo II

Genetic Toxicity Assessment 1

- Standard battery test data
  - AMES
  - In vitro chromosome damage/MLA
  - In vivo chromosome damage/MN

Mutations in *Salmonella typh TA102* by Ciprofloxacin

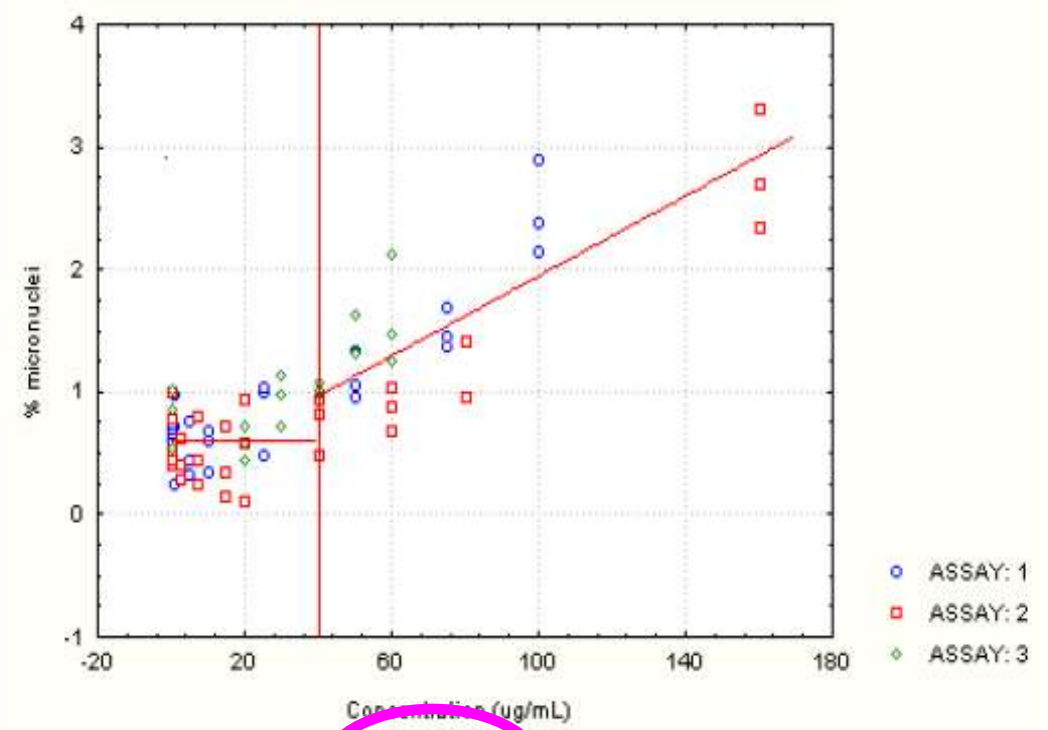


Data from Clerch et al. 1996, Env Mol Mutag 27:110-115

Ciprofloxacin MN induction in vitro

Lynch et al. 2003, Mutagenesis 18:345-353

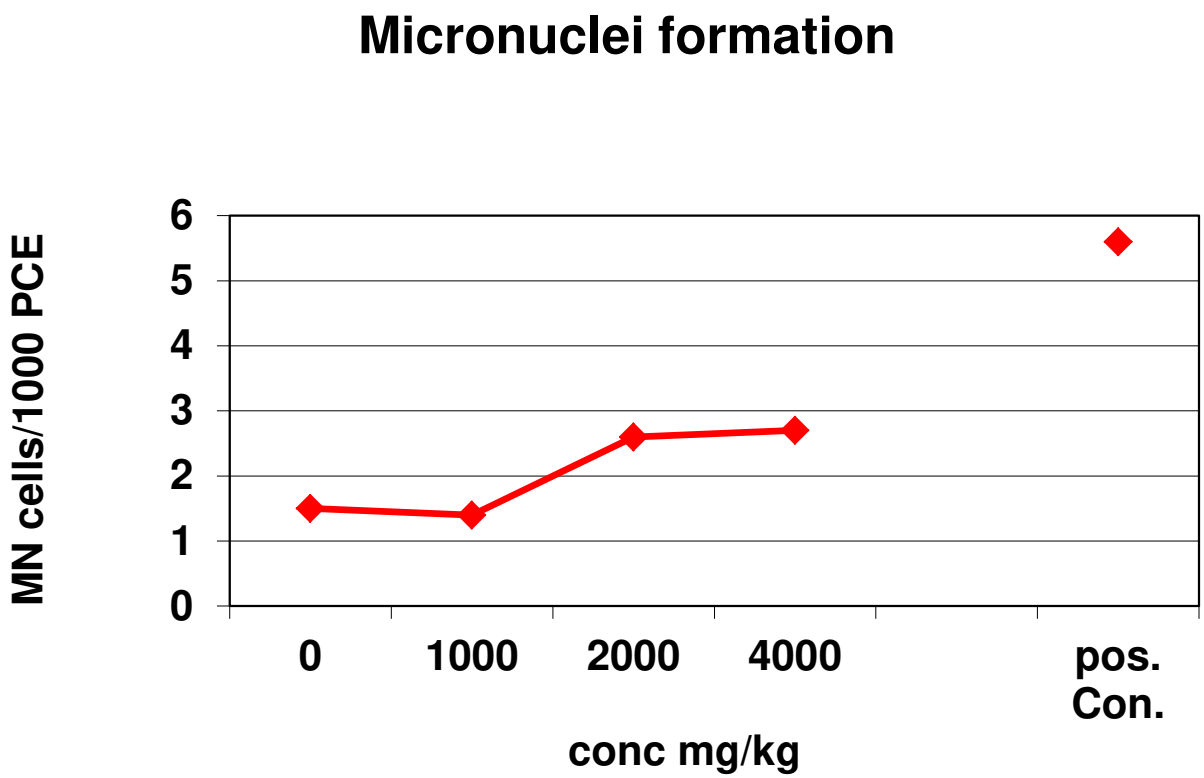
Micronucleus Test in Mouse Lymphoma Cells



The breakpoint was identified at conc.=40 ug/mL with %MN=0.636 fitted before the breakpoint and %MN=0.392+0.016*conc. fitted afterward.

Federal Institute for Drugs and Medical Devices

MN in vivo in NMRI mice in PCEs by Ciprofloxacin



Data from Herbolt et al. 2001, Mut Res 498:193-205

Standard battery result for ciprofloxacin

The standard battery result

bacterial mutagenicity	positive
MN in vitro mammalian cells	positive
MN in vivo rodent BM	negative

Standard battery positive
Knowledge on Mode of Action
nessecary for risk assessment

Mode of Action determination

- We need data to demonstrate
  - The lack of direct DNA reactivity
  - The mode of action leading to mutations
  - The target molecule/enzyme
  - The target molecule to be the key driver of mutation induction
  - The dose response to demonstrate a PoD (threshold)

Selectivity on target enzymes

Minimal Concentration to induce DNA-strandbreaks (cell-free assay)

	CC_{10} (µg/ml)		
Fluoroquinolone	Gyrase	Topoisomerase II	Factor
Pradofloxacin*	**0.005**	**4**	**800**
Enrofloxacin*	**0.005**	**6**	**1200**
Marbofloxacin*	**0.005**	**10**	**2000**
Danofloxacin*	**0.005**	**25**	**5000**
Orbifloxacin*	**0.005**	**25**	**5000**
Ciprofloxacin*	**0.005**	**10**	**2000**
Moxifloxacin*	**0.01**	**6**	**600**
Gemifloxacin*	**0.01**	**1**	**100**

*** Data from Koerber-Irrgang B, Dissertation, University Hamburg 2005**

Specific functional complex induction

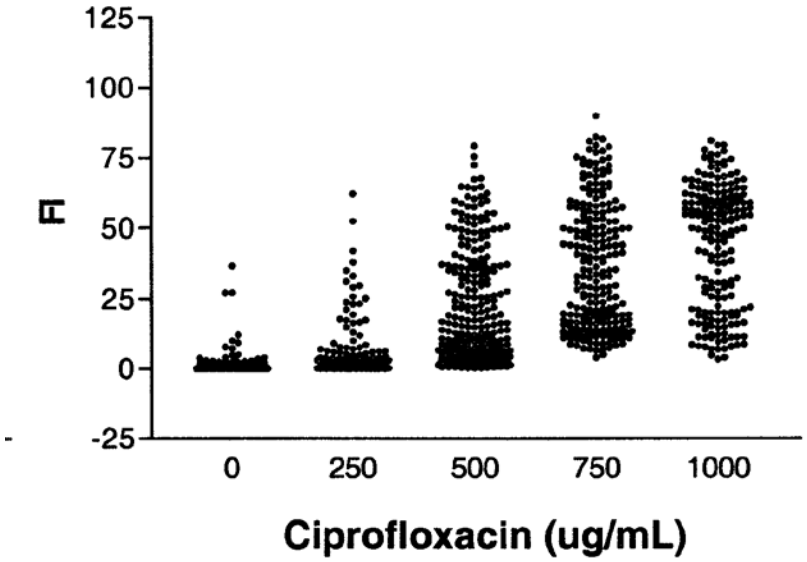
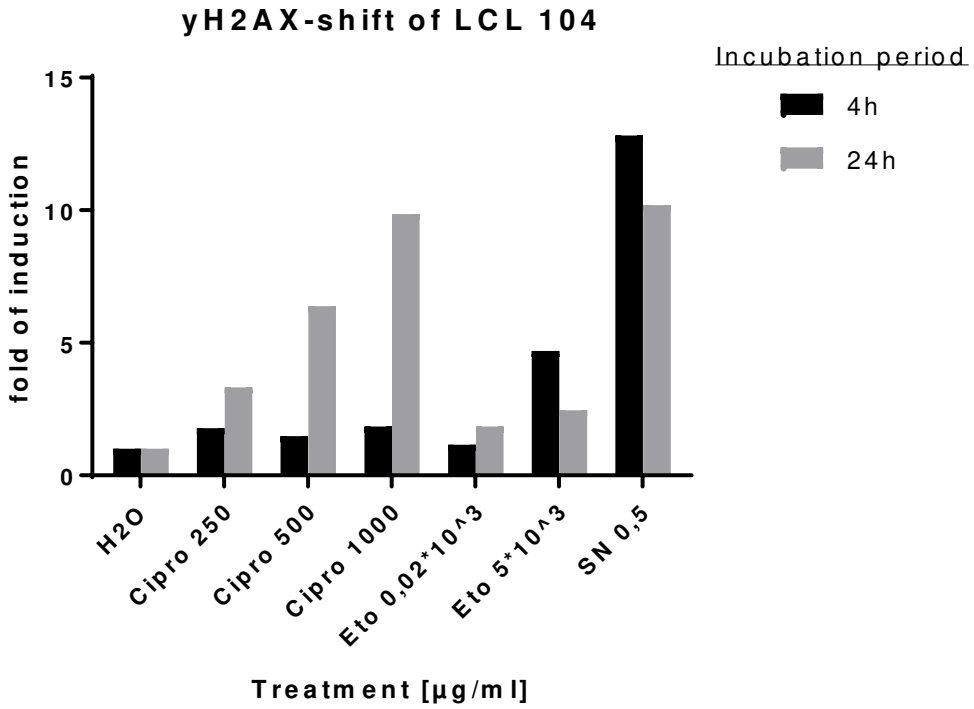


Fig. 1. TARDIS assay results in L5178Y mouse lymphoma cells. (**A**) Concentration-dependent increase in SCC formation with etoposide. (**B**) Time-dependent reversal of SCC formation following removal of etoposide (60 µg/ml). (**C**) SCC formation by known mammalian topoisomerase II poisons [doxorubicin (3 µg/ml), genistein (27 µg/ml), mitoxantrone (2.6 µg/ml) and ellipticine (1.2 µg/ml)] and two mammalian non-topoisomerase II poisons [aclarubicin (2 µg/ml) and

Lynch et al. 2003, Mutagenesis 18:345-353

Induction of yH2AX as measurement for DSB



Summary of ciprofloxacin

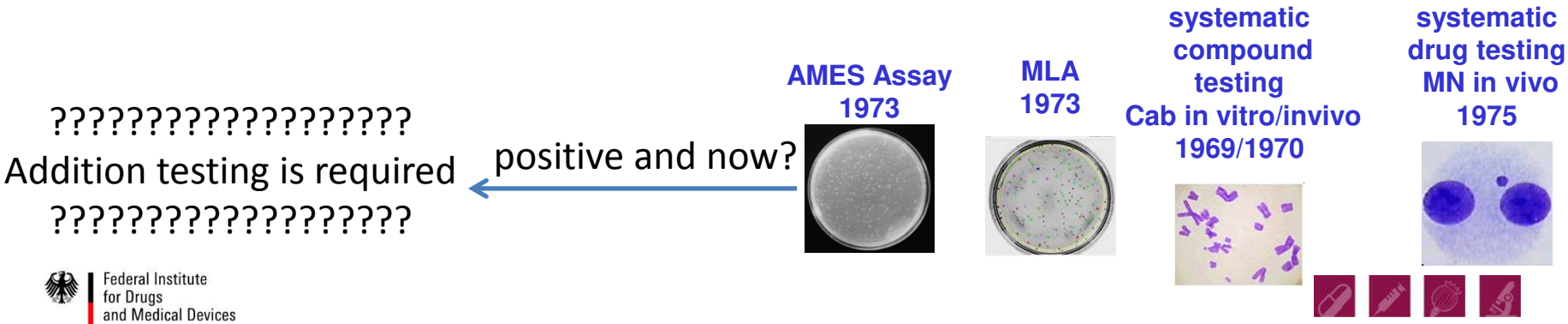
- Biochemical target is not DNA
- Hugh difference between effect on bacterial and mammalian target enzym
- Dose response of all assays show effects at high doses only

- Sufficient difference between human exposure and rodent exposure at doses with no genotoxic effect

Adverse outcome pathways – AOP – moving forward in MoA determination

- The MIE is the start of the an AOP
  - Knowledge of the dose response of the MIE
- The key events in the AOP
  - The more of the key events we know following the MIE
  - The more precise we may be to determine the complete dose response relationship between exposure and apical outcome

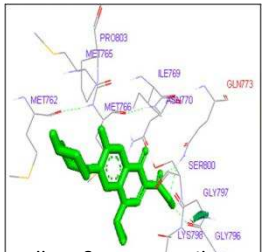
- This however is not possible with classical standard test systems:



Federal Institute for Drugs and Medical Devices

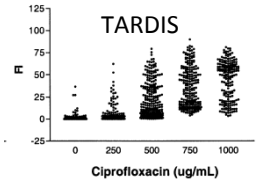
Potential AOP for catalytic topo II inhibition

MIE



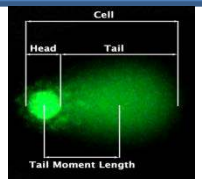
Jadhav & Karuppayil
In Silico Pharmacol. 2016

KCE1

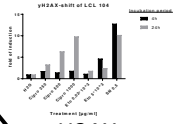


Lynch et al. 2003
Mutagenesis 18:345-353

KCE2

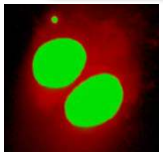


Comet assay

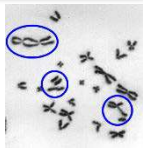


yH2AX

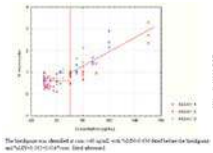
GAO

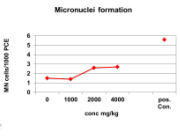


MN-assay



CAb-assay





Binding to Topo II ATP binding site

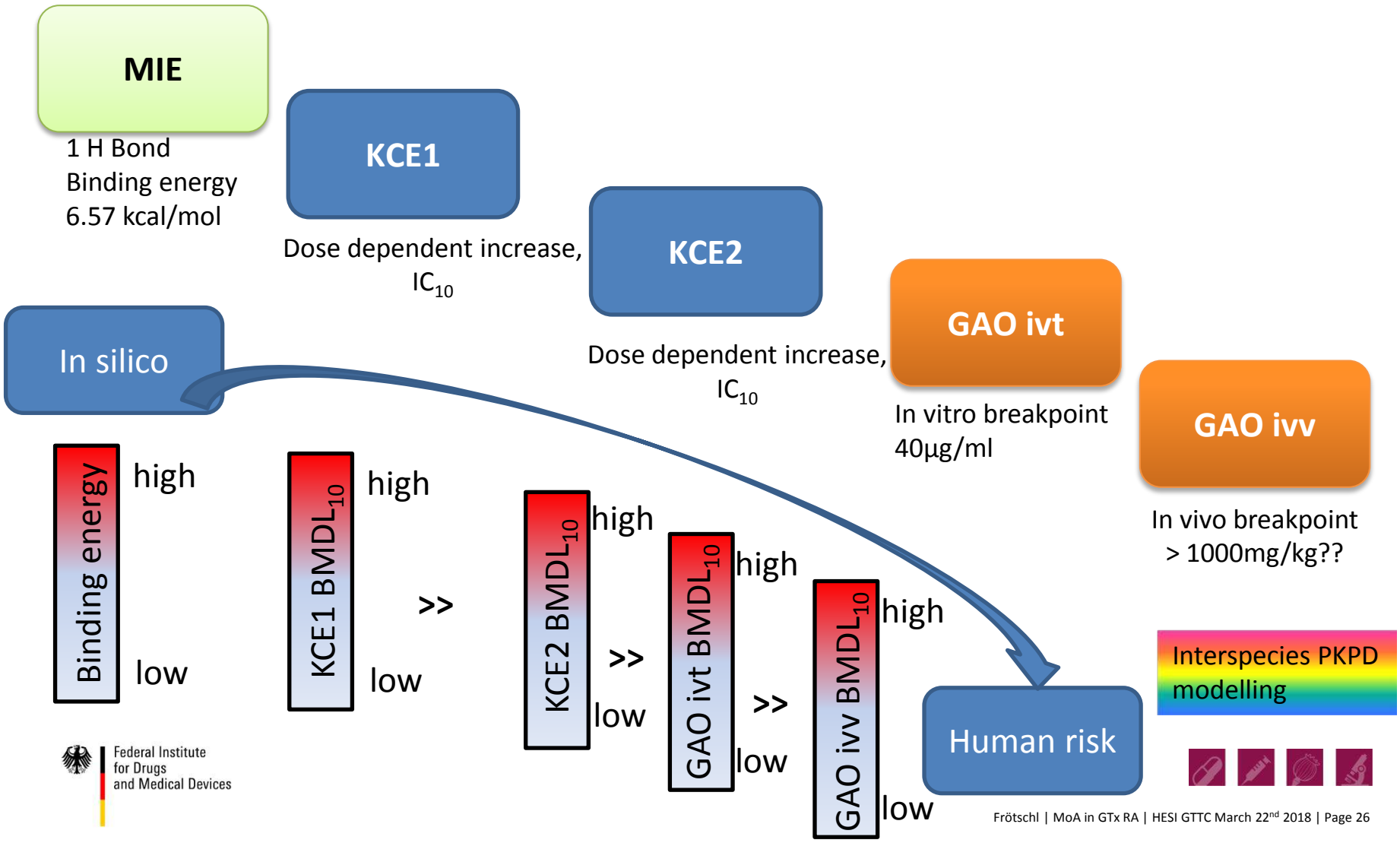
Stabilization of DNA-topo II complex inhibit religation activity

Blockade of the replication forks and transcription machinery

DNA double strand breaks

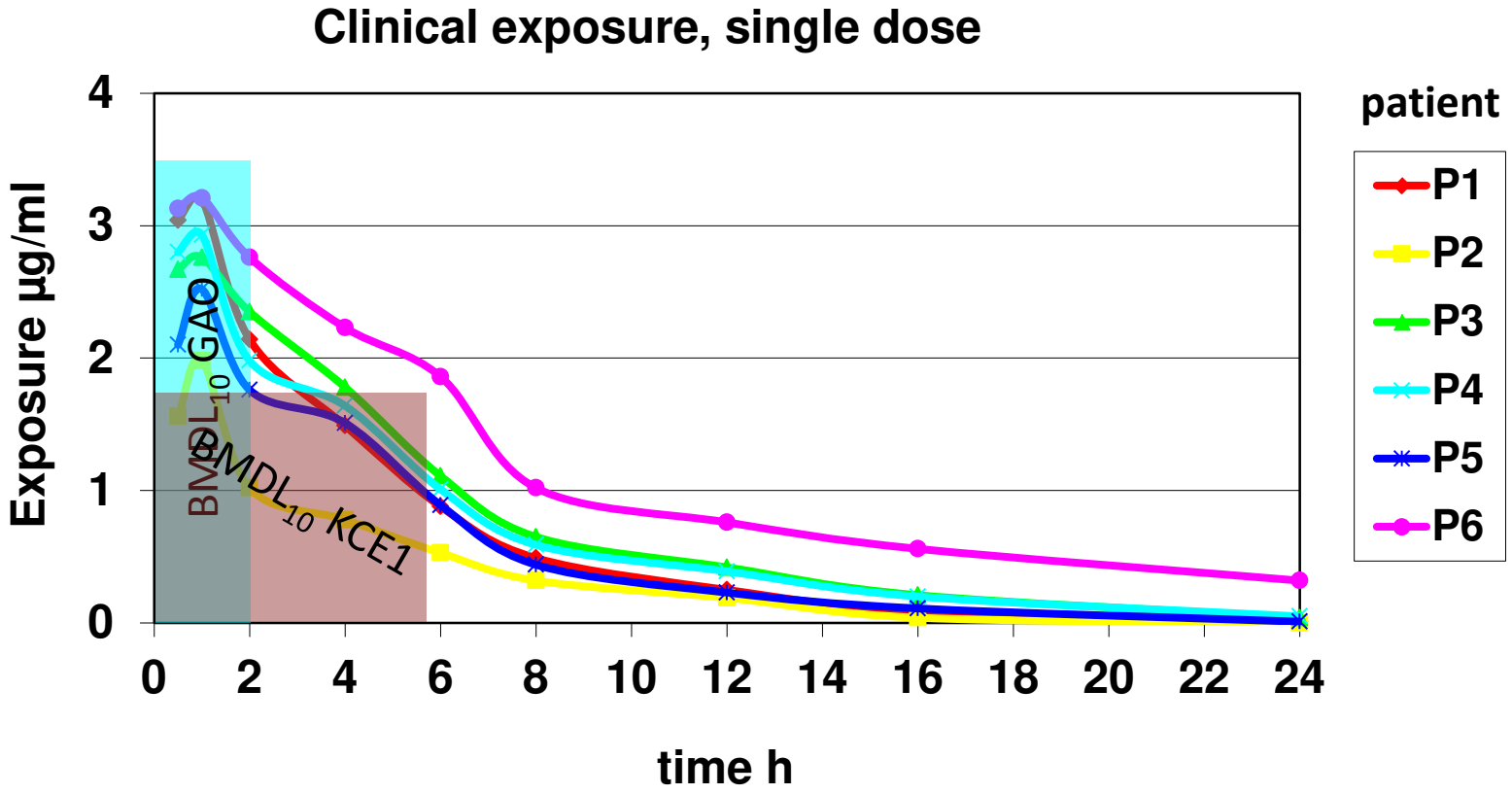
Clastogenic lesions

Dose response relationship between key events



Federal Institute for Drugs and Medical Devices

Risk assessment using dose response of AOP



Acceptability of this will depend on the level of uncertainty in the model

Federal Institute for Drugs and Medical Devices

From MacGregor et al. 2015 Mut Res 783:66-78

Conclusions

- Mode of action determination is crucial for genetic toxicity assessment of compounds positive in the standard battery
- Sufficient data on dose response relationship of genetic toxicity endpoints is needed for margin of exposure determination and acceptability of risk
- More informed MoA determination would potentially improve genetic toxicity assessment and also improve risk assessment
- AOPs for genetic toxicity pathways will provide guidance for MoA determinantion and help to improve experimental strategies in genetic toxicity testing
- Quantitative data on MIE and KCE in AOPs will support approaches in quantitative risk assessment in genetic toxicity

Thank you very much for your attention!

Contact

Federal Institute for Drugs and Medical Devices
Licencing Division 2
Genetic and Reproductive Toxicology
Kurt-Georg-Kiesinger-Allee 3
D-53175 Bonn

Contact person
Dr. Roland Frötschl
Roland.froetschl@bfarm.de
www.bfarm.de
Tel. +49 (0)228 99 307-3441
Fax +49 (0)228 99 307-5599

Federal Institute for Drugs and Medical Devices