Using Evidence-based Toxicology to Evaluate AOP

Thomas Hartung & CAAT team
Mechanistic & evidence-based toxicology
Current state of the art

Level of resolution

• Perturbed Molecular Network
• Molecular Pathway of Toxicity
• Toxicity Pathway / AOP
• Mode of Action
• Phenomenologic
AOP

• Narrative, low level of detail, existing info
• Biased by existing knowledge
• Not quantitative, no flux, no dynamics
• No QA / validation yet

PoT (Human Toxome)

• Molecular, high level of detail, emerging info
• Untargeted identification, causality
• Aiming for quantitative relations, fluxes
• Causality (to be shown)
The scandal of poor medical research

We need less research, better research, and research done for the right reasons
Why Most Published Research Findings Are False

John P. A. Ioannidis

“Basic research is like shooting an arrow in the air and, where it lands, painting a target.”

Homer Adkins, 1984

Nature 312, 212.

Food for Thought

Look Back in Anger – What Clinical Studies Tell Us About Preclinical Work

Thomas Hartung

Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA and University of Konstanz, CAAT-Europe, Germany
Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

“Fifty-three papers were deemed ‘landmark’ studies ... scientific findings were confirmed in only 6 (11%) cases. Even knowing the limitations of preclinical research, this was a shocking result.”
Believe it or not: how much can we rely on published data on potential drug targets?

Florian Prinz, Thomas Schlange and Khusru Asadullah

...data from 67 projects, ... This analysis revealed that only in ~20–25% of the projects were the relevant published data completely in line with our in-house findings... In almost two-thirds of the projects, there were inconsistencies between published data and in-house data that either considerably prolonged the duration of the target validation process or, in most cases, resulted in termination of the projects
This is why I do not believe in using existing knowledge without systematic review to form a point of reference.

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Look Back in Anger – What Clinical Studies Tell Us About Preclinical Work

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All models are wrong, some are useful.
George Box
Mechanism makes sense of signatures and separates the signal from the noise.

A good biomarker has a mechanistic foundation; Mechanism translates between model systems.

What we observe can be divided into:
- Signal
- Noise

what we see
The gift from validation to life sciences

- Validation of alternative tests is one of the rare examples of quality assurance in biomedical research (relevance, not only reproducibility)
  - “Evidence-based medicine goes in vitro!”

- OECD guidance document, how to apply Good Laboratory Practice in vitro
- Good Cell Culture Practice (minimal standards for academia)
- “Good Validation Practice” (OECD, ECVAM, ICCVAM, JaCVAM…)
- Publication Standards (ARRIVE, in vitro in preparation)
- Evidence-based Toxicology Collaboration (US & EU)
2006-7: Publication / 1st conference
Mar 2011: US EBTC
Oct 2011: Secretariat at CAAT
www.ebtox.com
Jan 2012: First conference hosted by EPA
Jun 2012: EU EBTC
Diverse working groups
Jul 2013: IUTOX, Seoul, Korea
Sep 2013: EuroTox, Interlaken, Switzerland
Systematic reviews increasingly embraced by EPA/IRIS, NTP and EFSA
21 Nov 2014: Forum Systematic Reviews, Baltimore
Limitations of current validation approaches:

• Time-consuming
• Non-systematic
• Focus on prediction of animal data

Advantages of an EBT Approach:

• Faster
• Systematic
• Can focus on mechanistic relevance
Valid(ated) models and reference substances

Pathway Identification

Proof of causality / predictivity

Human Toxome / AOP database

Pathway-based models

Proof of pathway coverage
Reproducibility

EBT

EBT

Mechanistically validated

Food for Thought ...
Mechanistic Validation

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Challenge: Quality Assurance of AOP

Based on
- Mechanism
- Evidence, i.e. systematic, objective, transparent
EBT and You

• Interested in
  – getting involved?
  – receiving updates?

• Get in touch!

• Thanks:
  – Marty Stephens
  – Sebastian Hoffmann
  – working groups

www.ebtox.com
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The chief aim of science is not to open a door to infinite wisdom, but to set a limit to infinite error.

Bertolt Brecht

In “Galileo”
Reserve

Possibly used in discussion
PROPOSAL FOR A TEMPLATE, AND GUIDANCE ON DEVELOPING AND ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS

Figure 1. A schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.
Limitations of animal models

• Humans are not 70 kg-rats…
• “One suit fits all”-models: tests can only be either sensitive or specific
• Statistically underpowered
• Too many endpoints without statistical …
• Rat vs. mice predictivity 60% for complex endpoints
• often 5-10x more false than real positives
Limitations of in vitro models

- Mycoplasma
- Dedifferentiation favored by growth conditions and cell selection
- Cells are bored to death
- Lack of oxygen
- Lack of metabolism and defense
- Unknown fate of test compounds in culture
- Tumor origin of many cells
- Cell identity

Cell models have not less limitations
Assessment tool for the quality of toxicological data

- Categorizes quality according to Klimisch scores
- Independent, but largely similar tools for in vivo and in vitro data/studies
- Expert advisory group
- 2 rater experiments:
  11 rater are applying the draft tool to 11 in vitro and in vivo studies
- Tool now available on the ECVAM website
- published Schneider et al. Tox Letters 2009, 189:138-144
- Impact for existing data for REACH
Evidence-based Toxicology for the 21st Century: Opportunities and Challenges*

Martin L. Stephens¹, Melvin Andersen², Richard A. Becker³, Kellyn Betts⁴, Kim Boekelheide⁵, Ed Carney⁶, Robert Chapin⁷, Dennis Devlin⁸, Suzanne Fitzpatrick⁹, John R. Fowle III¹⁰, Patricia Harlow¹¹, Thomas Hartung¹, Sebastian Hoffmann¹², Michael Holsapple¹³, Abigail Jacobs¹¹, Richard Judson¹⁴, Olga Naidenko¹⁵, Tim Pastoor¹⁶, Grace Patlewicz¹⁷, Andrew Rowan¹⁸, Roberta Scherer¹, Rashid Shaikh¹⁹, Ted Simon²⁰, Douglas Wolf¹⁴, and Joanne Zurlo¹

Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing

Richard Judson¹, Robert Kavlock¹, Matthew Martin¹, David Reif¹, Keith Houck¹, Thomas Knudsen¹, Ann Richard¹, Raymond R. Tice², Maurice Whelan³, Menghang Xia⁴, Ruili Huang⁴, Christopher Austin⁴, George Daston⁵, Thomas Hartung⁶, John R. Fowle III⁷, William Wooge⁸, Weida Tong⁹, and David Dix¹
Challenges in Applying EB Approaches to Toxicology

• Diverse study types in toxicology
• Availability of proprietary and negative data
• Limited nature of existing guidance
• Need for “buy in” on approaches & guidance to be developed
• “Publication” in databases versus scientific literature
• Are there enough studies by which to judge the performance of new methods?
• General resistance to change
• Misperception that evidence-based approaches leave no room for professional judgment