How to overcome limitations of new approach methodologies in the context of regulatory science

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Chemical Risk Assessment into this century

- **Animal toxicology** has been the major source of information, but now opportunities to accept **"alternative" approaches (***in vitro,* QSAR)

- New focus on the elucidation of the **details of mechanisms of toxicological action** to build **predictive models** (e.g., AOPs, Tox21)

Do Alternative Methods predict Apical Toxicity Endpoints ?

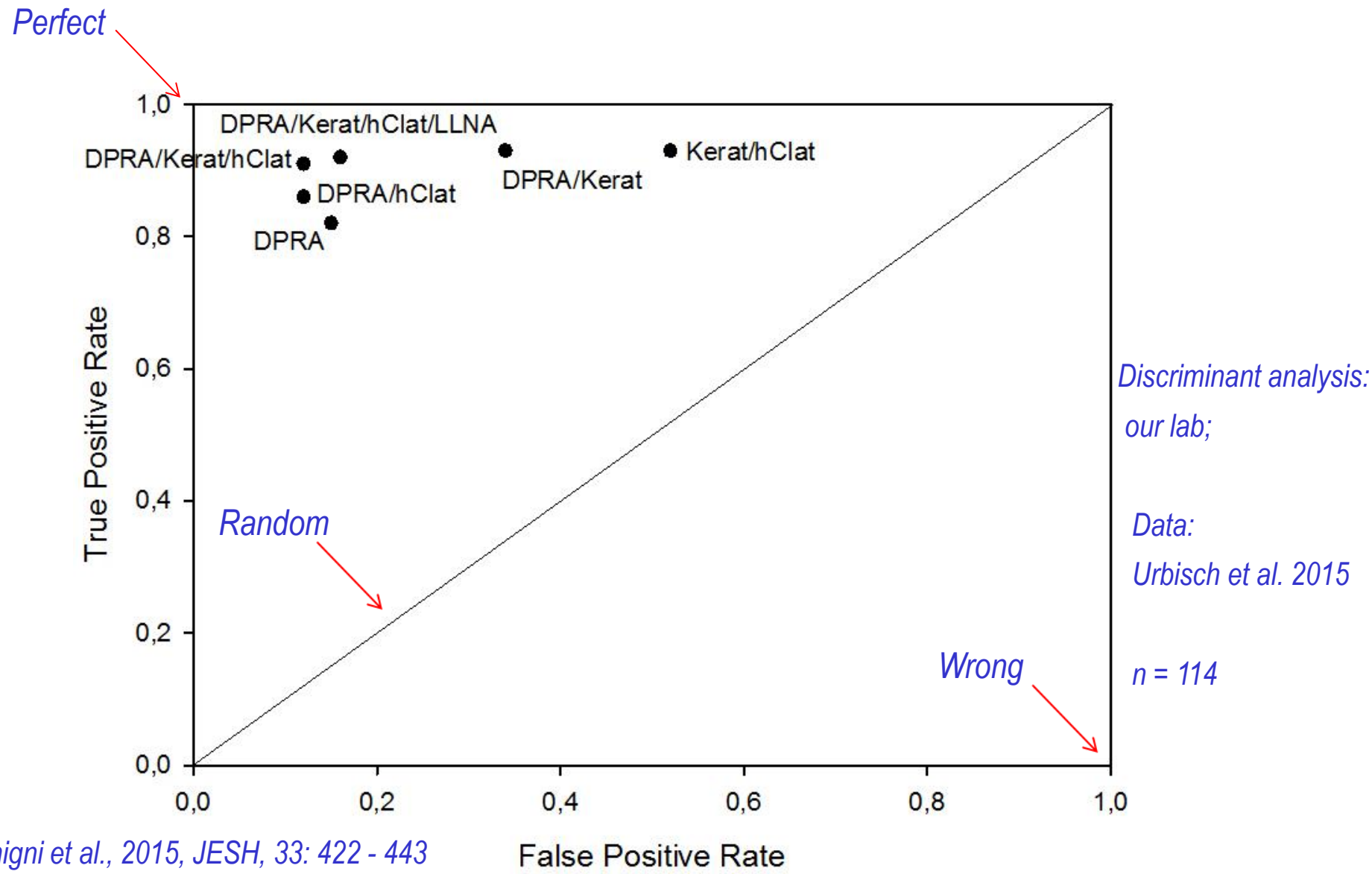
- **Regulatory issue**:

  present regulations are based on **apical endpoints**

- **Scientific issue:**

  correct predictions are the necessary **reality check f**or theories and hypotheses

Study I: **Skin Sensitization,** predicting **Human data**



Benigni et al., 2015, JESH, 33: 422 - 443

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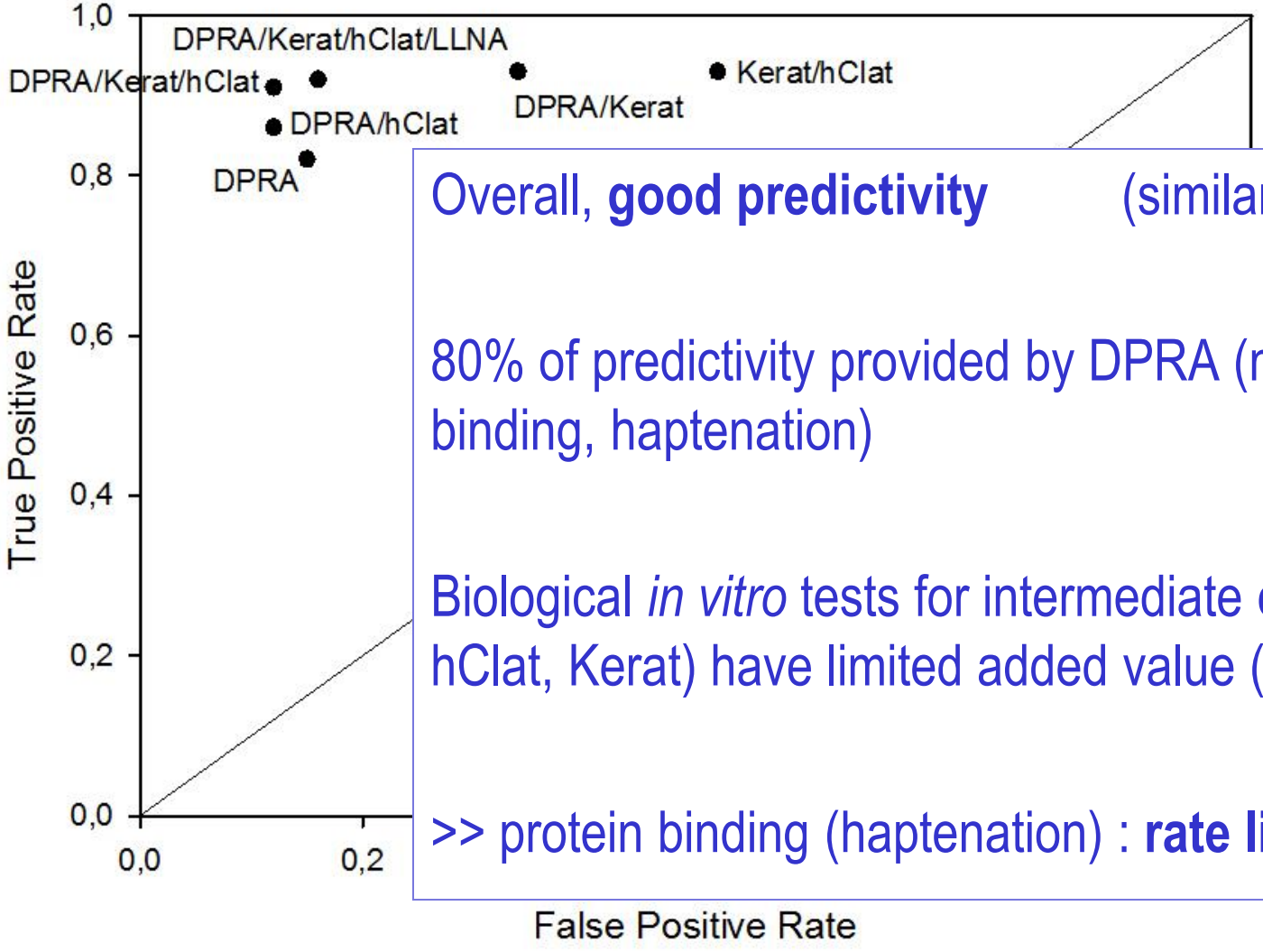

Overall, **good predictivity** (similar pattern for LLNA)

80% of predictivity provided by DPRA (model for protein binding, haptenation)

Biological *in vitro* tests for intermediate events (e.g., hClat, Kerat) have limited added value (5 – 10%)

>> protein binding (haptenation) : **rate limiting step ?**

Benigni et al., 2015, JESH, 33: 422 - 443

Study II. Predicting **rodent and human carcinogens:** an integrated testing strategy (tier)

Tier 1: **DNA-reactivity** (Salmonella and / or Structural Alerts)

If a chemical is negative ↓

Tier 2: **Tissue microarchitecture disorganization** (SHE Cell Transformation)

If a chemical is negative in both tiers ↓

Low probability of being a carcinogen

Benigni et al., 2013, Mutagenesis., 28: 107 – 116; *Benigni et al., 2013, Mutat.Res., 758: 56 – 61*

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Tier 2: Tissue microarchitecture disorganization (SHE Cell Transformation)

Predictivity:

rodent carcinogens: **90 – 95%** (125 / 130)

IARC human carcinogens (*1,2a,2b*)**:** **99%** (326 / 329)

*both **genotoxic and nongenotoxic** carcinogens*

Benigni et al., 2013, Mutagenesis., 28: 107 – 116; *Benigni et al., 2013, Mutat.Res., 758: 56 – 61*

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A complex endpoint modelled through a few rate limiting steps?

Study III. Toxcast assays *versus* Apical toxicity endpoints

- ***in vivo* endpoints** *(31 parameters / measures from Toolbox dbs)*

Carcinogenicity, Ames, *in vivo* Micronucleus, Acute, Subchronic, Repeated Dose, Reproduction and Development, Skin Sensitization, Endocrine disruptors

- **Toxcast assays**

n = 248, selected out of 913 assays (matching chemicals, etc…)

each *in vivo* test / parameter (n=31) *versus* each Toxcast assay (n=248):

>>>> 248 x 31 **≈ 1000 Spearman rank correlation coefficients**

Out of a floor of weak correlations, ≈ **80 correlation coeffs**.
statistically significant

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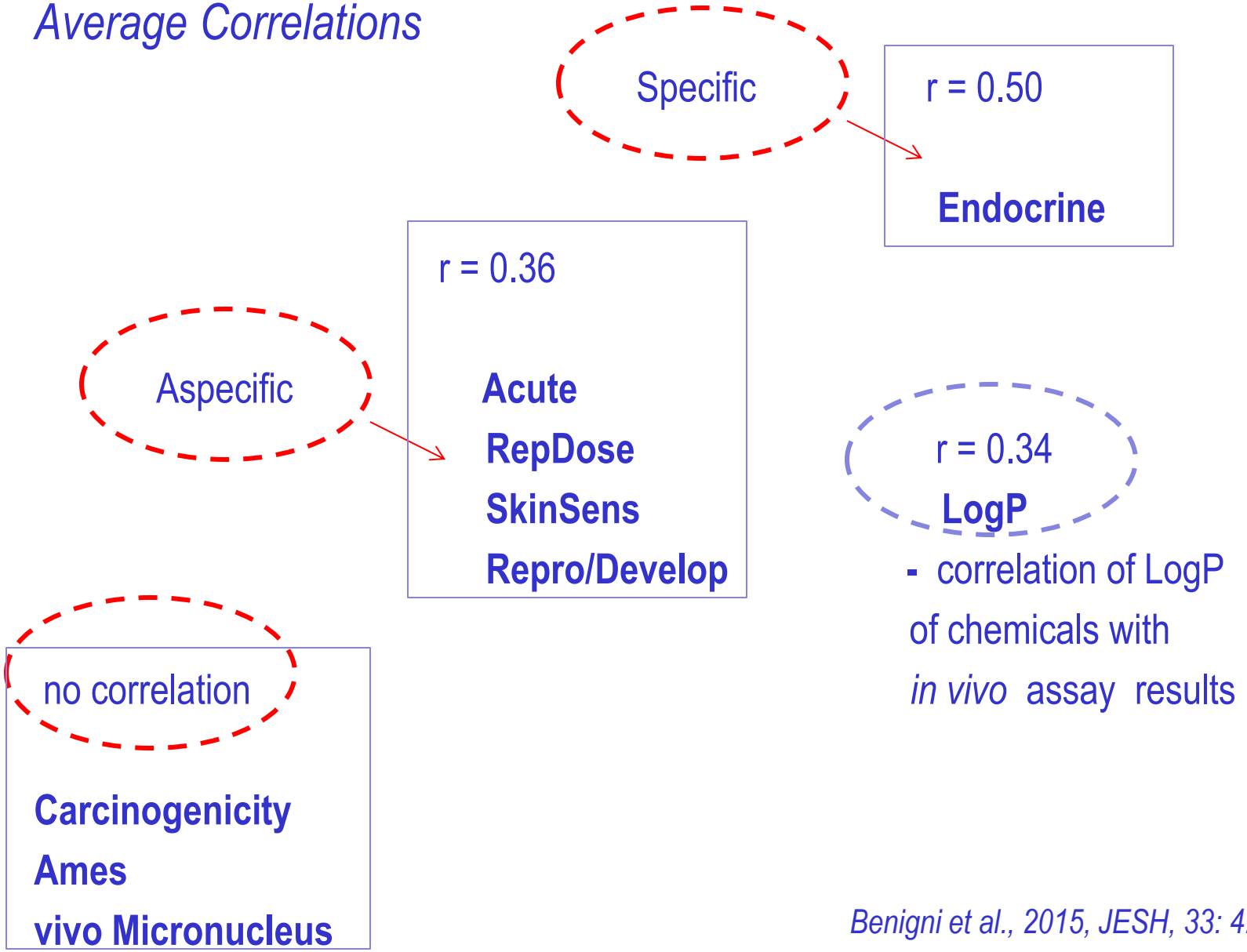
Study III. Toxcast assays *versus* Apical toxicity endpoints

Average Correlations



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Study III. Toxcast assays *versus* Apical toxicity endpoints

Improvement of Toxcast assays in progress (e.g., metabolism),

only **overall pattern of results important:**

- **Difficulties in modeling intermediate key events** (confirms Skin Sensitization results)

- ***in vivo:*** **continuum of events**, with **feedbacks**

  in vitro tests for intermediate events -in isolation- not a realistic model

- **Systemic toxicity endpoints (**e.g.,RepDose, Repro): **not well defined mechanistically**

Study IV. QSARs of Apical Toxicity Endpoints

- **Skin Carcinogenicity by Polycyclic Aromatic Hydrocarbons**

log Iball = 0.55 log *P* - 1.17 log(β 10logP + 1) + 0.39 LK + 0.47 HOMO + 1.93

log *P*0 = 6.67(± 0.217), log β = -6.81, *n* = 161, *r* = 0.845, *s* = 0.350, *F*1,155 = 12.8

Zhang, et al., 1992, Chem. Biol. Interact. 81: 149.

- **Carcinogenicity** (-/+) **by Aromatic Amines**

Canc = - 1.16 HOMO + 1.76 LUMO – 2.86 L(R) + 2.65 B5(R) + 0.40 MR3

+ 0.58 MR5 + 0.54 MR6 – 1.55 I(An) + 0.74 I(NO2) – 0.55 I(BiBr)

n = 66 (- =44; + = 73) Correct Classification = 87.9 %

Franke et al., 2001, Carcinogenesis, 22: 1561-1571

Study IV. QSARs of Apical Toxicity Endpoints

What do these QSARs mean ?

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Metabolic activation the rate limiting step ?

QSARs: Predictive models based on a few parameters.

Hansch, Chem. Rev. 2002, *102:* 783-812

The way to create a new and faster toxicology is still long, but progress is apparent. I.

- Some complex endpoints (e.g., skin sens, carcinogenicity) can be predicted with **satisfactory accuracy** by alternative methods

- **Compare with inherent variability, and predictivity** of animal tests towards human data

(e.g., LLNA versus Human: Accuracy = 0.82 n = 111)

LLNA versus Human Potencies: r = 0.76 n = 90)

The way to create a new and faster toxicology is still long, but progress is apparent. II.

Lessons learned

- Successful predictive models usually based on the quantification of only **one, or a few rate-limiting steps**.

- Rate-limiting steps: often **initiating events** of toxicological pathways.

- Markers for **intermediate events** have a **more limited correlation** with most endpoints.

The way to create a new and faster toxicology is still long, but progress is apparent. III.

- ….. quantification of only **one, or a few rate-limiting steps...**

- This contrasts with the current trend to dissect more and more the toxicity pathways

- … but confirms the general experience of building predictive models

"…In fields like ecology, systems biology, and macroeconomics, ***grossly simplified models capture important features*** *of the behavior of incredibly complex interacting systems…"* *Transtrum et al., 2015, J.Chem.Phys., 143: 010901.*

Empirical analysis of data is the only guide in the evolution of our understanding of how chemicals affect living systems

Corwin Hansch

- Types of toxicological pathways very different in nature:
  a **simplified linear chain inadequate** (feedbacks, intersections)

- **Biological plausibility not sufficient**

e.g., the dramatic story of the beta-caroten trial:

Beta-carotene: a cancer chemopreventive agent or a co-carcinogen? *Paolini et al. Mutat. Res. 2003 , 543:195-200.*

- Fill the operational gap between theory and implements:

Quantitative data analysis in the hands of experts in toxicology

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