Advancing the use of transcriptomic points of departure for regulatory decision-making

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US NIEHS: Scott Auerbach, Steve Ferguson
US EPA: Rusty Thomas
Toxicity testing is changing!

Toxicological Testing Paradigms

- **In silico** predictive toxicology
- **In vitro** transcriptomics
- High-throughput screening and targeted tests
- **In vivo** transcriptomics
- Conventional tests

All chemicals

Few chemicals
Challenge: How to efficiently analyze and interpret toxicogenomic (TGx) data?

Large gene lists

Complex analyses and interpretations

Use in risk assessment?
Vision for use of transcriptomics in regulatory decision-making

Large gene lists

Extract predictive signatures (biomarkers) and pathways

Dose-response modeling

At what dose do effects occur? Reverse dosimetry (IVIVE) required?

Risk assessment

Human exposure levels?

Align to AOPs

Oxidative DNA Damage, Increase

Inadequate Repair

DNA Strand Breaks, Increase

Mutations, Increase

Inadequate Repair

Chromosomal Aberrations

Hazard identification

Mode of action analysis
In the near-term: does a transcriptomic POD (regardless of hazard) provide protection from potential human health effects?
Context of use: various applications in regulatory decision making

Transcriptomic data set
- Extract predictive biomarkers
  - Dose-response modeling
  - Human exposure levels
  - Equivalent dose
  - Regulatory decision making

- Hazard
- MoA

- Thresholds of toxicity
- Transcriptomic point of departure

Reference dose

Context-specific regulatory applications
What are the regulatory concerns?

- Can we trust the new tools?
  - Validation – sensitivity, specificity, reproducibility, accuracy

- Will we miss toxicological effects?
  - Have we covered enough biology? Can we predict toxicological effects?

- Gene expression changes ≠ adverse phenotypic changes
  - Are we basing decisions on adaptive versus adverse effects?

- Gene expression changes are the first cellular responses
  - Will the dose at which we see responses be extremely low?
  - i.e., Are we being overly conservative?
    » Not feasible in terms of risk management

- What is the uncertainty associated with these new approaches?

- How do we do it (experience needed), who will generate the data, and will it give us comparable results?
Health Canada Research-Regulatory Collaboration
Why case studies?

- Building confidence
- Regulatory research feedback
- Harmonization and integration
- QA/QC Transparency
- Uncertainties and limitations
- Training and knowledge exchange
- Feasibility and challenges

Regulatory Adoption
Foundational studies: Most sensitive (lowest) pathway BMD provides a reasonable estimate of the PoD

Thomas et al., Tox Sci., 2013
Initial case study focus

- Traditional
  - MOA
  - Human relevance
  - POD

- Traditional & Toxicogenomics
  - MOA
  - Human relevance
  - POD

- Toxicogenomics
  - MOA
  - Human relevance
  - POD

Comparison
Hazard-agnostic tPOD within 10-fold (or less) of regulatory PODs (early DNA microarray studies)

<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Relevant Apical (mg/kd/day)</th>
<th>TGx PoD (mg/kg/day)</th>
<th>Apical: TGx</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Benzo[a]pyrene Liver</td>
<td>1.2</td>
<td>1.0 (lowest pathway)</td>
<td>&lt;2-fold</td>
<td>Moffat et al. Crit. Rev. Tox. 2014</td>
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<tr>
<td>Benzo[a]pyrene Lung</td>
<td>0.8</td>
<td>3.7 (lowest pathway)</td>
<td>~5-fold</td>
<td>Moffat et al. Crit. Rev. Tox. 2014</td>
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<tr>
<td>Furan (mouse) Liver</td>
<td>2.3</td>
<td>3.6 (median gene BMD)</td>
<td>&lt;2-fold</td>
<td>Jackson et al., Tox Applied Pharm. 2014</td>
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<td>Furan (rat) Liver</td>
<td>1.8</td>
<td>1.0 (median gene BMD)</td>
<td>&lt;2-fold</td>
<td>Dong et al. Arch. Tox., 2015</td>
</tr>
</tbody>
</table>

Similar conclusions from case studies on carbon black nanoparticles and acrylamide
Many questions remaining about how to select a POD that represents a tipping point for adverse effects
Which tPOD?

- Different gene sets identify similar tPODs to each other and to apical endpoint PODs.

**Approach**

- 1- 20 lowest pathway BMDs FDR P < 0.05, IPA enriched
- 2- 20 most significant pathways FDR P < 0.05, IPA
- 3- The lowest 20 pathways ANOVA P <0.05, BMD Data Viewer enriched
- 4- 20 biggest fold changes
- 5- BMD value between 25th and 75th percentile
- 6- Pathways with the highest number of connections to other pathways
- 7- 20 genes that contribute to the greatest number of pathways
- 8- Genes that are regulated by the top 20 most significant upstream regulators
- 9- Lowest pathway
- 10- Pathway BMD mean
- 11- Pathway BMD medians

Farmahin et al. Arch. Tox., 2017 (6 chemicals, 4 time points)
What if we use a hazard-based approach?

What if we used a different platform?

Impacts of more rigorous filtering?

MOA-specific pathway BMDs consistent with apical endpoint BMDs

- TGx BMD means are consistent across platforms
- TGx BMD means fall within interval between HCA and HCC
- Rigorous filtering had a small impact

Does this work for TGx biomarker gene sets? TGx-DDI biomarker BMC predicts the BMC of DNA damage

High-throughput CometChip® and TGx-DDI biomarker assay measured by TempO-seq in HepaRG cells.

Buick et al., Frontiers in Public Health, 2021

Li, HH et al. PNAS (2017)
But how do we know we’re not modeling noise?

- Need sufficient perturbations and rigorous filtering
- Gene sets to eliminate noise, or robust baseline required

Work done by Andrew Williams, Health Canada (channeling previous work by Scott Auerbach)

Methods:

- HepaRG cells
- solvent controls assigned randomly to 'dose groups'
- Run BMD analysis
  - BMR 1SD and default filters in BMDExpress
- Derive PODs
- Determine FDR of PODs

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Median # BMCs</th>
<th>25th Gene</th>
<th>Lowest Pathway</th>
<th>Median # BMCs</th>
<th>25th Gene</th>
<th>Lowest Pathway</th>
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<th>25th Gene</th>
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<td>0.07</td>
<td>0.06</td>
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<td>0.02</td>
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<td>0.07</td>
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<td>0.01</td>
<td>0.00</td>
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<td>0.03</td>
<td>2</td>
<td>0.00</td>
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</tbody>
</table>
Recent Research-Regulatory case studies to advance our vision
#1. Tiered testing for human health risk assessment
Hexabromocyclododecane (HBCD)

Objectives

• Gain experience in applying a tiered testing paradigm;
• Explore consistency across tiers;
• Evaluate use in risk assessment.

Gannon et al. *Food and Chemical Toxicology*, 2019.
Methods

Tier 1: ToxCast and Tox21 data

Tier 2: Rat liver RNA-sequencing

- Male and Female Fischer F344 N = 10

- Dietary concentrations of 250, 1250, and 5000 mg HBOD/kg diet.

- Altered pathways, upstream regulators

- Signatures of toxicity

Tier 3: Rat sub-chronic studies

- 0 days

- Start test diet 5 females per dose per strain and 5 male F344 per dose

- Body weight and food consumption measurements

- 7 days

- Body weight and food consumption measurements

- 14 days

- Body weight and food consumption measurements

- 21 days

- Necropsy all rats; body weight and food consumption measurements

- 28 days

- Blood samples; tissues harvested, weighed and assessed for gross morphological changes

Gannon, Moreau, Farmahin et al. Food and Chemical Toxicology, 2019.
Transcriptomics is highly consistent with the other tiers for hazard ID

Confirmed effects observed *in vivo*
- Hundreds of differentially expressed genes

Identified sex-specific effects
- More changes in males than females

Genes were associated with pathways suggesting:
- Alterations in metabolism of xenobiotics and nuclear receptor activity, oxidative stress, cell proliferation and apoptosis, metabolism of glucose and lipid, immune response, fibrotic activity, and hormonal balance

Transcriptomic biomarker analysis revealed
- CAR and PXR biomarker activation at all doses in both sexes (no other biomarkers)
BMD analysis reveals bi-modal distributions and consistency between males (A) and females (B)

<table>
<thead>
<tr>
<th>Approach used to derive BMD</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td>Median of significantly enriched pathway BMDs</td>
<td>77</td>
<td>73</td>
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<tr>
<td>20 genes with the largest fold changes</td>
<td>84</td>
<td>65</td>
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<tr>
<td>Lowest statistically significant pathway</td>
<td>66</td>
<td>71</td>
</tr>
<tr>
<td>Lowest overall pathways (5% and min 3 genes)</td>
<td>7.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Tier 2 is highly overlapping with Tier 3

- human oral equivalent doses for ToxCast AC50s & rat liver transcriptomic BMDs, compared to apical endpoint BMDs in rats and relative to human exposure (Canadian Health Measures survey)

Gannon et al. *Food and Chemical Toxicology*, 2019.
#2. PFAS regulatory needs

- Understand potential toxicity and potencies of emerging PFAS
- Acceptable concentrations of PFAS in drinking water and for cleanup of contaminated sites
- **Prototypes for comparison – PFOA and PFOS**

## Methods

- 10 concentrations, 4 time points (1, 4, 10 and 14 days)
- Media changed every three days and cytotoxicity monitored

Rowan-Carroll et al., Tox Sci 2021
Median gene BMC (central measure of activity)

Potency comparison of prototypes: PFOS > PFDS > PFOA > PFBS

- PFBS – Least potent
- PFOS – Most Potent
- PFOA
- PFDS

Rowan-Carroll et al.
Tox Sci, 2021
Similar potency rankings in overall BMC distribution

Potency: PFOS > PFDS > PFOA > PFBS

- Lowest effects occur at similar concentrations for PFOS, PFOA, PFDS (similar potencies)
- Transcriptional activity initiated: 1 – 15 µM
- PFOS has more genes fitting BMC models below 20 (biological activity)
- Potential use of liver toxicity thresholds (Ramaiahgari SC et al. Tox Sci 2019)
tPODs for PFOA and PFOS consistent with apical PODs and potential for human health risk

- Lowest pathway
- 5th percentile gene
- Conventional tests
Decreasing BMC with increasing PFAS chain length

- Analysis separated by functional groups
- Relationship between chain length and potency
- Use of information for read-across to inform data-poor and untested PFAS

Reardon et al. Tox Sci, 2021
#3. Bisphenol and bisphenol replacements: tPODs to compare potencies and identify active/inactive chemicals

- Estrogenic activity and potency analysis of BPA alternatives
- MCF7 cells, 9 concentrations, 48 hr exposures

Parodi-Matteo *in preparation*
#3. Bisphenol and bisphenol replacements: tPOD approach identifies active/inactive chemicals and enable potency ranking

Rooney et al.  
*Chem Res Toxicol.* 2021

<table>
<thead>
<tr>
<th>Chemical</th>
<th>ERα Prediction</th>
<th>25th gene</th>
<th>Lowest Pathway Median</th>
<th>IPA ER Median</th>
<th>ERα BM Median</th>
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<td>BPA</td>
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<td>0.0018</td>
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<td>BPAF</td>
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<td>4,4'-BPF</td>
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<td>BTUM</td>
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Log_{10} BMC (μM)
Gene set enrichment analysis of genes fitting BMC models (concentration-responsive genes) reveals high similarity across the chemicals.
What does this mean?

• Dose at which we see transcriptional perturbations (i.e., tPOD) in short-term studies predicts the dose at which adverse effects occur following longer-term exposures.

• tPODs are generally conservative, but not overly conservative.

• A variety of approaches work, both hazard-based and agnostic.
  • When the transcriptome is robustly perturbed, prolonged exposed at this dose is likely to lead to adverse health consequences.

• Approach taken should be context specific.
  • Selecting the lowest tPOD is protective of adverse health effects.

• Case studies useful for informing regulatory applications and building confidence.
Major needs

• Socialize this idea
  • Paradigm-changes are challenging

• Establish best practices for deriving tPODs for different contexts of use
  • OECD Transcriptomic Reporting Framework has a BMD module, which ensures transparency in regulatory submissions and may facilitate developing acceptable practices

• Identify model-specific baseline filtering requirements

• Studies to establish confidence that hazard-agnostic tPOD can be protective of human health effects

• Demonstrate applicability across broad chemical and biological space
  • Critical to mainstream integration for decision making

• Determine how to address uncertainty
These case studies build confidence in the application of tPODs in regulatory evaluations and help to define suitable contexts of use

- Much to learn from focused collaborative studies on individual chemicals or small chemical groupings
- Demonstrating applicability across broad chemical and biological space will be critical to mainstream integration for decision making
- Growing interest in use across regulatory bureaus

### Conclusions

### Potential uses for toxicogenomics in risk assessment

<table>
<thead>
<tr>
<th>Program</th>
<th>Weight of evidence</th>
<th>Mode of Action analysis</th>
<th>Prioritization</th>
<th>Chemical Grouping to support Read across</th>
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<td>Food</td>
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<td>Biologics and genetic therapies</td>
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<td>Marketed health products</td>
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