Application and use of ‘omic’ data for next generation risk assessment:
From constructing adverse outcome pathways to defining points of departure

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OBJECTIVE APPLICATION OF OMICS FOR NGRA

NOTEL
- Dose response relationship and identification of biologically relevant dose

MoA
- Determination of Compounds primary MoA through pathway analysis using GSEA
- Underpinning AOP key events and MIEs

Biologic al RA
- Comparison of differentially expressed signatures from one treatment to a database of previously reported gene signatures or to another sample.
OUR FOCUS

• Focus on exposure driven, non-animal approaches for consumer and environmental safety risk assessment
• Data required for safety decision should be driver
• Dose response information is essential
• Understanding the underpinning biology

• We are not looking for a way to do the animal test without the animal
RISK ASSESSMENT

Consumer use scenario

Internal exposure assessment

*In vitro* and *in silico* tools to identify and interrogate pathways

Evaluate responses

Risk assessment

Confidence in off-target effects
A TIERED APPROACH FOR RISK ASSESSMENT

Tier 1: *In silico* MIE prediction
- QSARs
- Docking models
- MIE Atlas

Tier 2: Pathway Identification
- Transcriptomics
- In-vitro screening panels
- High content imaging

Tier 3: Pathway Characterisation
- 3D organotypic models
- Systems biology models
- MD simulations

Adopting an **exposure-driven** risk assessment approach

Weight of Evidence

Mechanistic understanding
Application of transcriptomics has held a lot of promise for risk assessment and has yet to deliver. Ever increasing data sets – 10,000s of data points but need to be able to infer which are relevant for the decision verses being biological noise.

- Techniques for data generation are continually changing.
- Techniques for handling and processing the data are continually evolving and are known to impact on the interpretation of the analysis.
- Defining key drivers within pathways and networks is a challenge.
- Impact for qualitative use vs impact for quantitative assessment.

Starting to see several applications coming through where looking at define a subset of genes for classifying compounds against a particular endpoint. Eg. GARDA. —primarily binary classification but now moving towards potency.
NOTEL

NOTEL is essentially the derived dose (or concentration) of a compound or stressor that does not elicit a meaningful change in gene expression (i.e., the threshold of the dose/concentration that elicits minimal mechanistic activity).

- Provide broad coverage of biological space
- Define sensitivity to estimate a point of departure in relation to other test systems including apical endpoints
- Significant ongoing efforts in developing method as new high throughput technologies become available that make dose response analysis across a broad dose range feasible and cost effective

Thomas et. al., Tox Sci, 2013

Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment
Farmahin et al. arch Tox 2017
NOTE – BMD EXPRESS 2.0

Dose response data provides key information to associate increasing changes in gene expression with the defined treatment.
Enables an estimate of potency in case of chemical and dose at which limited/no perturbations occur.
Recommended minimum number of doses is 3
APPLICATION SCENARIO
- STILL IN DEVELOPMENT

Exposure due to consumer use (mg/kg/day) from PBPK modelling

In vitro threshold concentration (uM) measured as free media concentration

Free plasma concentration (uM) corresponding to consumer use from PBPK modelling

In vitro to in vivo extrapolation and POD comparison
BIOLOGICAL SPACE

- Is technology sensitive or too conservative
- Breadth of coverage of biological pathways
- Biological sample coverage. Complexity of surrogate test system
- Acute vs chronic responses
What are the consequences of pathway perturbation in Humans?

What pathways are perturbed? E.g. NrF2

What pathways are associated with this disease state in Humans?

What are we trying to avoid?

ADVERSE OUTCOME PATHWAYS

Chemical interaction with target

Cellular response

Organ response

Organism response
Characterize molecular mechanisms in idiopathic pulmonary fibrosis (IPF), a chronic form of lung disease with an unknown etiology characterized by fibrosis of the interstitium of the lungs as a proxy for bio-persistent induced lung fibrosis (Selventa)

Utilise algorithms such as Reverse Causal Reasoning to understand mechanistic causes of gene expression changes.

APPROACH: REVERSE CAUSAL REASONING

Data Set → State Change Analysis

Modeling → Knowledgebase

Causal Reasoning → List of Hypotheses

Hypotheses Are Incorporated into Networks → Causal Network Model

Hypothesis Investigation
DEVELOPING A DATA DRIVEN AOP

Identification of mechanistic causes leading to differential gene expression changes

Reverse Causal Reasoning

Knowledgebase
A collection of cause and effect relationships

Differentially expressed genes

Stress Responses Lead To Apoptosis

- Response pathways activated to deal with ER stress, oxidative stress and DNA damage are not sufficient to ameliorate the insult, resulting in apoptosis.
  - EIF2AK3, FOLR1 and SMARCA4 may be novel mediators of ER stress, DNA damage and epithelial cell apoptosis in IPF.
    - EIF2AK3/PERK activation reduces protein synthesis and enhances apoptosis (PMID: 10588420)
    - FOLR1 is the folate receptor, and folate deficiency leads to impaired DNA repair and increased DNA damage (PMID: 9882578)
    - SMARCA4 is a positive regulator of TP53 activity (PMID: 11950834)

For Example:
- exp(L8)
- exp(TN)
- exp(ANKH)
- exp(S)
- exp(T)

Lung Epithelial Cell Apoptosis

- Response to virus
  - LPS from A. fumigatus
  - Cytotoxic T cell activation
  - Natural killer cell activation
  - catol(TLR3)
  - catol(TLR4)
  - catol(CCR5)
  - catol(CD11c)
  - catol(PI)3
  - catol(IT)2
  - catol(BRCA1)
  - catol(P53)
  - catol(NFκB Complex)
  - catol(CEBPδ)

- Macrophage differentiation
  - CEBPε
  - CEBPβ
  - CEBPα
  - CEBP γ
  - CEBP δ

- Type II macrophage activation
  - IL-1
  - IL-6
  - IL-1β
  - IL-13

- response to wounding
  - IL-1
  - IL-6
  - IL-1β
  - IL-13

- Inflammatory response
  - IFN-γ
  - TNF-α
  - IL-1β
  - IL-6
  - IL-13
  - MIP-1α
  - INF-γ
  - IL-2

- CD40L
  - CD86
  - CD80

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Primary aim can defining similarity across gene signatures aid in reducing uncertainty in a read-across argument
Support similarity in mode of action
GOVERNANCE AND EXTERNAL DATA STANDARDS – CASE STUDY OMICS

Provision of Guidance has been and is ongoing to ensure recognised standards are met for use in risk assessment. A study can be truly reproducible when it satisfies at least the following three criteria.

- All methods are fully reported.
- All data and files used for the analysis are available.
- The process of analyzing raw data is well reported and preserved.

1. For Every Result, Keep Track of How It Was Produced
2. Avoid Manual Data Manipulation Steps
3. Archive the Exact Versions of All External Programs Used
4. Version Control All Custom Scripts
5. Record All Intermediate Results, When Possible in Standardized Formats
6. For Analyses That Include Randomness, Note Underlying Random Seeds
7. Always Store Raw Data behind Plots
8. Generate Hierarchical Analysis Output, Allowing Layers of Increasing Detail to Be Inspected
9. Connect Textual Statements to Underlying Results
10. Provide Access to Scripts, Runs, and Results

Modified from https://www.r-bloggers.com/what-is-reproducible-research/
http://www.reproducible-bioinformatics.org/
GOVERNANCE OF OMIC DATA

Standards → Ease of Use → End user driven

Traceability → Flexibility

Framework for Data Curation & Storage

- RNAseq Differential Expression
- Microarray Differential Expression
- Whole genome methylation Analysis

Framework for Data Processing, Analysis & Automation

Framework for Data Interpretation
SMART FILES AND PROVENENCE
DECISION POINTS AND TRANSPARENCY OF DATA ANALYSIS STEPS

standardized reporting frameworks or templates for appropriate data, associated metadata, and analytical processes and transparency in the data processing methods used.

Framework for Data Curation & Storage

Framework for Data Processing & Analysis

Framework for Data Interpretation

Functional group (pathway/Gene set) & Gene Level BMD identification for NOTEL
SUMMARY

• Transcriptomics has progressed and is still developing.
• Omics data can be utilised for risk assessment and handled in a manner that is both consistent and similar to the current needs of risk assessors.
• Utility for defining POD as well as MoA and also to aid in a weight of Evidence decision for read across arguments.
• Good practice needed to ensure transparency, and reproducibility of not only data generated but analysis.
• Should not limit future developments
THANK YOU!