Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing

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Introduction

To ensure that New Approach Methodologies (NAMs)-based Next Generation Risk Assessments (NGRA) are sufficiently protective of human health, it is key to ensure the NAMs are fit-for-purpose. This is especially challenging for Developmental And Reproductive Toxicity (DART) where there are multiple endpoints or health effects and not one AOP can be used as a backbone for determining the relevant outcomes. To this end, the mechanistic and biological coverage, as well as gaps, of NAMs for DART were assessed, based on the overall mechanisms involved in human reproduction and embryo-foetal development.

Methods

Guided by the overall knowledge of human reproductive biology and embryo-foetal development, the key stages and morphogenetic events (**Fig 1**) were considered for individual targeted literature searches. Standardised query terms were used in the EPA-developed Abstract Sifter literature search tool, results were subjected to a quality check and validation, key biomarker terms from the final set of results for each of the searches were enriched and extracted using the TERMite recognition engine (SciBite) and 3 vocabularies, i.e. GENEBOOST and miRNA from SciBite and a bespoke one for DART-related Biological Processes (DrBP). Terms that exceeded set thresholds were pooled to generate 3 master lists of genes, miRNA and biological processes, respectively. These master lists were used for determining the biological coverage of DART NAMs [High Throughput Transcriptomics (HTTr); Cell Stress Panel (CSP); *In Vitro* Pharmacological Profiling (IPP), ReproTracker®; and devTOXquickPredict™) and assess the gaps remaining.

Results

A total of 103,607 articles served as the comprehensive pool from which biological marker terms relevant to reproductive and developmental mechanisms, referred to as Developmental and Reproductive Signalling (DARS) markers, were extracted (**Fig 1**). These included 3,551 DARS genes, 474 DARS processes and 338 DARS miRNAs. Genes and processes alone were used for coverage and gaps analyses in this work.

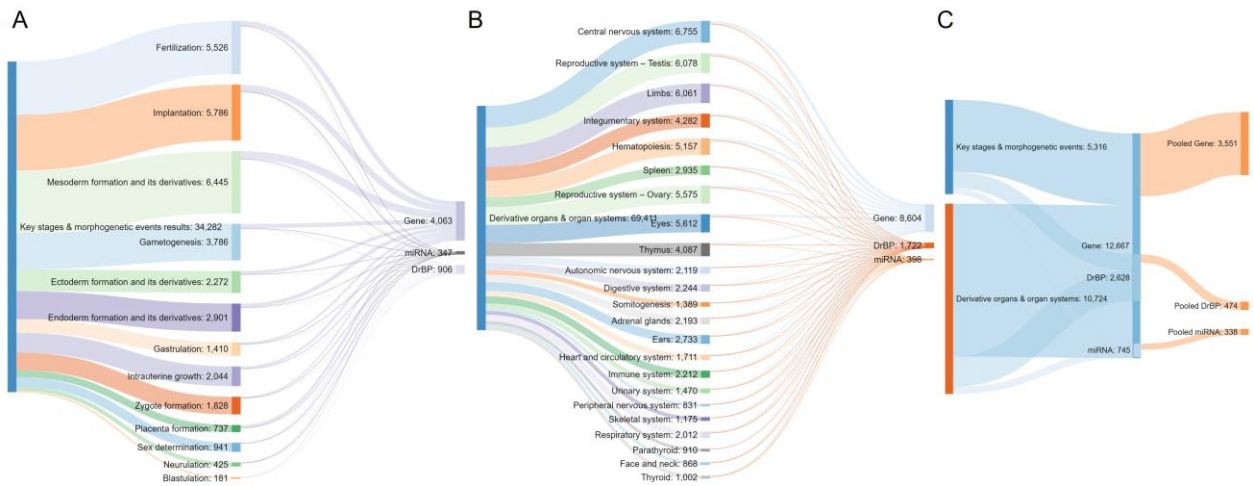

Figure 1. Sankey diagram indicating the number of articles screened for each stage (A) and organ type (B), the number of stage (A) or organ-specific (B) sets of DARS markers extracted and pooled sets of DARS markers (C)

Coverage: Baseline gene expression was determined for the 3 cell lines currently core to HTTr analysis in our NGRA framework; HepG2, HepaRG and MCF-7, and the undifferentiated human induced pluripotent stem cells (iPSCs), as it is used in both the ReproTracker® and devTOXquickPredict™ assays. 2,730 out of the 3,551 genes were found present in the gene set from these 4 cell lines (**Fig 2**). The 474 DARS identified biological processes could be broadly classed into categories depending on their role in cellular processes (**Table**). Most of the general cellular and functional processes are expected to be covered through cell survival or cytotoxicity read outs. About 13% of the receptor or enzyme activity related biological processes are covered by the IPP assays. Some aspects of specific differentiation and metabolic signature of teratogenicity are captured in the ReproTracker® and devTOXquickPredict™ assays, respectively. Signalling was covered in the analysis of genes and genotoxicity out of scope of the DART framework.

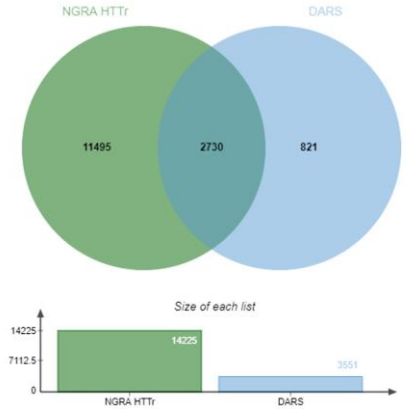

Figure 2. Coverage of DARS genes by NGRA HTTr cell lines (HepG2, HepaRG, MCF-7 & hiPSCs)

Gaps: 821 out of the 3,551 genes not seen in the 4 cell lines represented the protein classes (Panther classification system) of GPCRs, helix-turn-helix (HTH) transcription factors and intercellular signalling molecules . Some gaps also remained in the specific cellular and functional processes as well as specific differentiation processes.

Category	Examples
General cellular process	Signalling, DNA methylation, Cell differentiation
Specific cellular process	Myelination, Embryonic cleavage, Cytokine secretion
General functional process	Cell migration, Tight junction assembly, Cell motility
Specific functional process	Sperm motility, Neuron migration, Macrophage migration
Specific differentiation	Neurogenesis, Hepatocyte differentiation, Cardiocyte differentiation
Receptor or enzyme activity	PI3-kinase activity, MAP kinase activity, FGFR activity
Signalling pathway	Notch pathway, Nodal pathway, Hippo signalling
Cellular stress	Oxidative stress, Heat-shock response, Apoptotsis
Genotoxicity	Cell cycle checkpoint, Mitotic DNA replication checkpoint, DNA integrity checkpoint

Table: DARS identified molecular process categorised depending on the cellular function

Conclusions

- A master list of DARS markers was generated by systematic categorization of reproduction and development into key stages and targeted literature search.
- Between the 4 cell lines and IPP panel, almost 80% coverage of DARS genes was determined
- Higher tier and/or bespoke testing may be required to address tissue-specific or temporal mechanisms, lacking in the current NAMs.

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