PREDART: Prediction of human developmental and reproductive toxicity through non-mammalian assays

PREDART aims to:

- Explore and understand the key pathways which underlie normal development and the perturbations which drive developmental toxicity in non-mammalian systems (e.g. zebrafish, *C. elegans*, slime mould, embryonic stem cells);
- Extrapolate the findings in the surrogate systems to mammalian teratogenic potential;
- Develop stable, medium-throughput test systems to provide an early indication of developmental toxicity.

BACKGROUND

Developmental and reproductive toxicity studies in rats and rabbits form the main basis for regulatory assessment of the potential effects of chemicals on the developing foetus. These studies use large numbers of animals, are expensive and time consuming. In addition, the relevance to humans of effects seen in these studies is not always clear and significant species differences exist[1]. In addition, there remains a dearth of information about the fundamental pathways of embryogenesis and the effects of toxicity on them.

If a compound is found to be toxic to reproduction and/or development, this has significant consequences for potential use of the product, including restriction from occupational and/or consumer use or prohibition of authorisation for any use under regulatory schemes, including REACH and the EU's plant protection products regulation. It is therefore important to obtain an indicator of developmental toxicity early in the product development pipeline.

In the past the use of alternative models in lower species or *in vitro* systems has been hampered by the drive for a 1:1 predictive screen for mammalian outcomes. However, in the absence of an understanding of the complex underlying developmental pathways and species differences this has proved to be unachievable.

In recent years numerous alternative developmental biology model systems have been explored, ranging from whole organism systems including zebrafish, *Caenorhabditis elegans* and slime moulds, to cell based systems using mouse or human embryonic stem (ES) cells[2]. Groups working with these systems are embracing and exploiting the explosion in molecular biology and genetics to better understand the underlying pathways driving normal embryogenesis and the effects of perturbations to these pathways.

The key to unlocking the potential of these alternative test systems is to understand the conservation of key pathways across different species, including mammals, and to use these non-mammalian (surrogate) systems to inform mammalian toxicity potential. The issue of exposure concentrations in the test systems versus those in rats/rabbits and humans will also need to be assessed. There has

Sponsors

Shell, Syngenta

Budget per project

Phase 1: Up to £100,000 inc. VAT where applicable

Phase 2: Up to £750,000 inc. VAT where applicable

Key words

Developmental and reproductive toxicity, pathway perturbations, mammalian and surrogate species, molecular biology, non-mammalian assay

been progress in this direction in recent years, of which Carney and colleagues provide an excellent review[3], but there is now a need for a focused effort towards practical, commercial solutions, which will include an integration of the available systems.

3Rs BENEFITS

A standard two-generation reproductive and developmental toxicity study typically uses around 2,500 test animals. An early indication of developmental toxicity potential during compound development (such as relevant pathway perturbations) would be of benefit as a screening or ranking tool, helping to inform compound selection and direct testing strategies. The information can also be used to predict structure activity relationships (SAR) and steer chemistry design away from these key alerts.

This could impact on animal use in a number of ways including:

- Reducing the number of regulatory toxicity studies carried out in animals (e.g. developmental and reproductive toxicity, teratogenicity, carcinogenicity) on compounds with potential for developmental toxicity that would not therefore be progressed into product development.
- Supporting weight of evidence and/or read across arguments for registration, consequently reducing and replacing the use of animals for developmental toxicity testing. In the longer term as our understanding of underlying pathway perturbations improves and confidence is gained in these models, the opportunities to replace the use of rodents and rabbits will increase.

NEED FOR COLLABORATION

Developmental and reproductive toxicity studies in academia have traditionally focussed on specific specialist areas (e.g. specific species and specific phenotypic outcomes e.g. ossification, organogenesis, egg hatching), whilst in industry the focus has tended to be on one to one predictive models.

Bringing together a range of disciplines (e.g. biologists, biochemists, molecular biologists, and bioinformaticians) and sectors (e.g. academia, SMEs and industry) will maximise the integration of these foci and the possibility of solving this Challenge. Not only due to leverage of their collective expertise, but also by bringing together various perspectives to a high profile, relatively long standing issue.

OVERALL AIM

To develop and characterise non-mammalian assays that can provide an indication of developmental and reproductive toxicity potential to mammals, including man, in order to reduce and refine *in vivo* mammalian testing.

KEY DELIVERABLES

Phase 1

- Exploration of key developmental pathways that are involved with cell migration and differentiation in the non-mammalian developmental test systems (e.g. C elegans, slime mould, zebrafish) and ES cells, combining currently available published data and new data from a limited set of test compounds with known outcomes;
- Overlay the information on key developmental pathways from the different test systems and identify common denominators.
- Comparison of findings with genomic and other data from existing mammalian studies (including ossification, organogenesis, palatal development), to identify conserved pathways across the phyla which could be exploited as surrogate markers for mammalian developmental toxicity potential;

Phase 2

- Further assessment of key developmental pathways in the combined test systems using a range of test compounds and comparison of findings with genomic and other data from existing mammalian studies.
- Identification of biomarkers for developmental toxicity that can be measured in lower order species and embryonic stem cells;
- Determination of the applicability domain (i.e. the types of chemical structures, physicochemical properties and mechanisms of action for which the model can make reliable predictions).
- Assessment of compound uptake by non-mammalian systems in relation to physico-chemical properties;
    - Key characteristics are reproducibility, robustness and reliability;
    - Assessment needs to include comparison with *in vivo* data;
    - For physico-chemical properties Lipinski and Tice rules need to apply (Delaney *et al*. 2006; Lipinski 1997, Tice 2001);
- Transfer to a stable medium-throughput system with commercial potential.

IN-KIND CONTRIBUTIONS

Phase 1

- The sponsors can provide input into the initial compound set and appropriate existing *in vivo* data for comparison;
- Guidance on key industry requirements for model development and incorporation into existing testing strategies.

Phase 2

- The sponsors can provide appropriate additional test compounds and where possible, existing animal testing data for assessment and validation of the test system(s).
- The companies can also provide expertise and connections to networks and other research programmes with which they are involved.

REFERENCES

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