CrossDART: multi species *in vitro* developmental toxicity testing

Overall aim

1. The aim of this Challenge is to develop and qualify an *in vitro* approach that can reliably predict early or surrogate indicators of teratogenicity of pharmaceutical drug candidates.

Duration

2. Phase 1: up to six months, Phase 2: up to three years.

Budget

3. Phase 1: up to £100k, Phase 2: up to £1M.

Sponsors

4. Sponsors define the Challenges in collaboration with the NC3Rs to set out the business case and 3Rs benefits, with a view to using the product developed. Sponsors are required to provide in-kind contributions to help solve the Challenge.

5. The Sponsors for this Challenge are AbbVie, AstraZeneca, Bayer AG, Boehringer Ingelheim, LEO Pharma A/S, Merck Healthcare KGaA, Medicines for Malaria Venture, Novartis and Roche.

Background

6. Drugs intended for the treatment of women of child-bearing potential (WoCBP) must be tested for teratogenicity – the potential for the drug to cause harm to a developing embryo or fetus (1). Embryo-fetal development is a complex process which involves coordinated biological events that are susceptible to external influences. Exposure of the mother to a teratogenic drug can disrupt the normal formation of organs and may result in a wide range of malformations.

7. Studies to assess the potential teratogenicity of a drug typically involve evaluation in pregnant animals in two species (a rodent and non-rodent) as specified in the International Council for Harmonisation (ICH) S5 (R3) guideline (1). These embryo-fetal development (EFD) studies are generally conducted on the drug candidate once it has entered clinical development, but the animal models are not always predictive of humans and often do not provide mechanistic insight (2). Several alternative models exist that are used to

predict teratogenicity during the selection of candidate drug molecules, but improved models are needed to address the current limitations and permit their broader use:

- *In silico* QSAR models have predictivity limited to defined chemical spaces (3, 4).
- *Ex vivo* embryo assay cultures (e.g. using rodent, rabbit) and non-mammalian models such as *Caenorhabditis elegans* or zebrafish may have limited mechanisms in common with human development (5-15).
- *In vitro* stem cell-based models (4, 16-29):
    - Rely on adherent monolayer cultures or disorganised 3D structures, both of which lack the spatiotemporal and morphological context of the developing embryo.
    - Can only recapitulate certain aspects of embryo development and do not cover mechanisms such as neurulation and the effect on the trophoectoderm/placenta.
    - Do not provide an accurate method to extrapolate *in vitro* culture concentration to pharmacokinetic (PK) parameters of drug exposure in humans.
- Qualification of human *in vitro* models is hampered by the availability of human data and the limited relevance of animal data.

8. There is a need to develop improved *in vitro* assays both in human and in preclinically relevant species (e.g. rat, rabbit, non-human primate (NHP)) to aid with translation and understanding of species-specific effects and human relevancy.

The Challenge

9. The aim of this Challenge is to develop and qualify an *in vitro* approach that can reliably predict early or surrogate indicators of teratogenicity of pharmaceutical drug candidates. The proposed approach should cover as many mechanisms of embryo development as possible. To support validation and translation, the approach must cover human as well as selected preclinical species used for EFD studies (rat, rabbit, NHP).

3Rs benefits

10. A standard EFD study requires multiple pregnant females (80 to100 for rodents and rabbits; and 48 for NHPs). In the case of EFD findings, additional mechanistic studies requiring further animals might be performed to identify if the findings are relevant to human. The packages of drugs approved by the FDA currently include around 67 EFD studies per year (around 6,000 animals) (30). For biopharmaceuticals which are not pharmacologically active in rodents, the EFD study can be replaced by an enhanced pre- and postnatal development (ePPND) study in NHPs.

11. This Challenge has the potential to deliver 3Rs benefits in the pharmaceutical industry by:

- Providing more predictive approaches that can be used for early screening and derisking to prevent drug candidates with teratogenic potential from progressing into animal studies.
- Use in mechanistic studies to investigate species differences/species-specific effects and human relevancy especially in the case of equivocal EFD results.
- Replacing *in vivo* studies for drugs which have a mode-of-action that is suspected to adversely influence morphogenesis, as specified in the ICH S5(R3) guideline.
- In the longer term, once qualified and accepted by regulatory authorities, substituting or deferring EFD studies in one of the two species required.

12. The assays developed through this Challenge will also have applicability to the food, chemical and agrochemical industries where teratogenicity assessment is also required.

Key deliverables

13. The aim of this Challenge is to deliver a panel of *in vitro* assays that provide early or surrogate indicators of teratogenicity of drug candidates. Assays are expected to be *in vitro*, but *in silico* models that complement the *in vitro* assays are in scope.
    - The *in vitro* assays should:
        - Cover both human and at least two preclinical species used in EFD studies (rat and rabbit must be included; NHP is desirable).
        - Cover as many mechanisms of embryo development as possible, (but note recapitulating full development is not in scope).
        - Be qualified using reference compounds and compounds provided by the Sponsors.
        - Be suitable for early screening (e.g. able to screen ten compounds/month, require less than one gram of compound and be amenable to automation).
14. The Challenge should also deliver a computational prediction model to integrate and analyse the data generated from the assays which would determine the teratogenicity potential of individual compounds. This should factor in dose and efficacy data/predictions to estimate the therapeutic window where feasible.

What is not in scope:

15. The following approaches are not in scope for the Challenge:
    - Assays on non-mammalian organisms, tissues or cells.
    - Human and NHP embryonic stem cell-based models.

- Whole and synthetic embryos.
- Models that only assess placental barrier disposition.
- *In silico* approaches alone.

Phase 1 deliverables

16. The deliverables for Phase 1 are:

- Select appropriate *in vitro* assays for human and one preclinical species (rabbit or rat).
- Initial establishment, characterisation and optimisation of the assays, and evaluation of:
    - Required endpoints (such as, but not limited to, morphological parameters, genotypic profiles).
    - Throughput, to allow subsequent upscaling as needed.
    - Reproducibility.
- Perform initial small-scale qualification with a limited panel of positive and negative reference compounds.
- A plan for, and initial development of, the computational prediction model to determine the teratogenicity potential of individual compounds.

Phase 2 deliverables

17. Phase 2 includes essential and desirable deliverables. **Essential deliverables** are:

- Scale the model to include species not covered by Phase 1 (rat, rabbit and potentially NHP).
- The assays must be at least medium throughout and amenable to automation to be used for early screening of drug candidates.
- Extensive characterisation and validation/qualification of human and animal *in vitro* assays:
    - Follow ICH S5 guideline for qualification of alternative assays (1).
    - Test positive and negative reference compounds (complete ICH S5 reference compound list (1) and Sponsor compounds).
    - Comparison of data to traditional EFD *in vivo* models and existing *in vitro* assays to assess predictivity.
- Methods to extrapolate drug concentrations used in the assays to maternal PK exposure parameters *in vivo*.

- Full development of the computational prediction model to determine the teratogenicity potential of individual compounds.
- Engagement with regulatory authorities to gain feedback with the help of the Sponsors.

The application must include a plan to commercialise the results into a product or service.

18. The Challenge also includes **desirable deliverables** that are intended to maximise the potential of the model in the longer term. The desirable deliverables are:
    - Assessment of intra-/inter-laboratory reproducibility.
    - Applicants are encouraged to consider:
        - The spatiotemporal and morphological context of the developing embryo.
        - Maternal drug metabolism and exposure.
        - The potential to factor in efficacy data for therapeutic index estimation.

Sponsor in-kind contributions

19. The Challenge will be supported through the provision of in-kind support from the Sponsors. The in-kind support offered includes:
    - Scientific expertise.
    - Input on end-user requirements.
    - Compounds to test and associated *in vitro*/preclinical data (to extend validation of the assay to a broader drug space/modalities).
    - Bioinformatics support (analysis/modelling).
    - Support with regulatory interaction.
    - Support for agreed sample analysis (e.g. drug concentration assessment).

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