CRACK IT

Title of Challenge: Neuratect

Background

Detection of neurotoxicity and seizure liability induced by chemicals and pharmaceuticals presents a major scientific challenge due to the physiological and morphological complexity of the central nervous system (CNS). Regulatory authorities such as the Organisation for Economic Co-operation and Development (OECD, 2007) and the US Environmental Protection Agency (US EPA, 1998) along with ICH guidance for the pharmaceutical industry (ICH S7A, 2001 and M3 (R2), 2009) rely on animal studies in their regulatory requirements. Neurotoxicity studies, including developmental neurotoxicity studies, are required for new chemicals and for CNS drugs in development. CNS drugs also undergo the seizure assessment for safety pharmacology.

For neurotoxicity studies, assessment of potential functional impairments is performed using *in vivo* test methods with associated neuropathological endpoints. Example tests include:

- Neurobehavioural deficits (e.g. Functional Observational Battery (FOB)/Irwin).
- Alterations to learning and memory (e.g. Water maze, T/Y-maze).
- Loss of sensory and motor function(e.g. startle response, gait analysis, grip strength).

Seizure assessment for safety pharmacology is performed using a stepwise approach starting with ex *vivo* assays followed by *in vivo* models.

These studies are not always predictive of adverse events in humans, are costly and time consuming and not amenable to high throughput testing of chemicals and compounds.

Advances in technology and questions around the utility of animal models, as well as associated welfare concerns, have resulted in scientists looking for better and more predictive alternatives for toxicological testing of new compounds that minimise animal use in assessing the potential impact of consumer products on the neurological system.

In particular, recent developments in (3D) cell cultures and techniques applied to explore stem cell differentiation have demonstrated the potential to develop complex tissue structures *in vitro*. There is increasing evidence that these 3D culture systems can capture important components of the complex physiology of a tissue or an organ better than classical monolayer approaches (Pampaloni *et al*., 2007; Lee at al., 2009). For example, a human neuronal stem cell line derived from umbilical cord blood (HUCB-NSC) has been used to test developmental neurotoxicity using morphological and structural endpoints (Buzanska *et al*., 2009).

Recent studies have described the culture and interrogation of primary and human induced pluripotent stem cells (iPSCs) neurones for the assessment of various parameters of neurotoxicity (Novellino *et al*., 2011, Robinette *et al*., 2011, Ylä-Outen *et al*., 2010). However, as yet, there is no integrated, human relevant, *in vitro* 3D system for assessing neurotoxicity or seizure liability based on the inherent physiological characteristics of the neurological system (i.e. electrophysiological, tissue architecture, neuronal cell type) that is fit for purpose for use in the commercial sector. The rapid progress in the use of human iPSCs and innovation in the supporting technology platforms provide the opportunity to develop a dynamic, human-relevant strategy to screen for neurotoxicity and seizure liability potential *in vitro*.

3Rs benefits

- Regulatory assessment of neurotoxicity and seizure liability relies solely on *in vivo* testing. To carry out these studies for one compound currently requires several hundred rodents and costs approximately £1 million (Smirnova *et al*., 2014).

- A physiologically-relevant 3D *in vitro* iPSC-based model could replace the significant number of animals currently used to assess the potential neurotoxicity and seizure liability induced by chemicals and pharmaceuticals.

Need for collaboration

A wide range of expertise is essential to solve this Challenge including experts in neurophysiology, pharmacology, toxicology, iPSC technology and bioengineers. Industry Sponsors will provide expertise in system requirements and support for testing and validating the system where appropriate.

Overall aim

The aim of this Challenge is to generate physiologically-relevant human stem cell-based model(s) to identify neurotoxicity (neuronal viability/functional impairment) *in vitro*. For this purpose, it is ideally expected that the platform will integrate morphological/structural endpoints with electrophysiological parameters.
The same platform should also be translatable to safety pharmacology applications to assess seizure liability, in order to replace the current gold standard *ex vivo* hippocampal slice assay.

Key deliverables

A successful platform would require the use of small quantities of compound and cells and have a high-throughput capability consistent with its use in identifying the potential neurotoxicological effects of large numbers of test substances. Ideally, the optimal platform should permit the integration of morphological/structural endpoints with functional (i.e. electrophysiological) endpoints.

Phase 1

- Development of a cell culture platform that produces a mixed population of iPSC neurons which represent the different types of neuronal cells physiologically present in the brain.

- Demonstration of cell maturity through, for example, appropriate patterns of spontaneous firing.

- The mixed population should represent, for example:
  - Neurons (excitatory, inhibitory and interneurons)
  - Glia
  - Astrocytes

- Proposal of a set of acceptance criteria for neuronal viability and function based on morphological, pharmacological, structural and electrophysiology readouts.

Phase 2

Development of a cell culture format that:

- Supports the 3D growth and development of a mixed population of mature iPSC neurones.

- Permits the collection of morphological and structural endpoints.

- Is amenable to electrophysiological recording (e.g. individual action potentials and / or local field potentials).

Delivery of an integrated platform that has been used to:

- Identify and validate electrophysiological endpoints using reference compounds and morphological and structural endpoints using known neurotoxicants and non- neurotoxicants provided by the Sponsors.

- Identify and validate electrophysiological endpoints using reference compounds and morphological and structural endpoints using known seizurogenic and non-seizurogenic compounds provided by Sponsor/s.

- Commercialisation of a validated, 'fit for purpose' platform made available to scientists across the bioscience sector.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Applicants are not required to:

- Perform extensive regulatory validation studies.

Sponsor in-kind contributions

Phase 1

- Intellectual input (e.g. requirement of suitable test systems or translation from *in vitro* to *in vivo* modelling).

- Expert advice on appropriate electrophysiological measurements.

Phase 2

- Provision of a reference training set and of neurotoxicants/seizurogenic compounds.

- Relevant *in vitro* and *in vivo* preclinical and clinical data as available*.

Duration

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

Budget

Phase 1: up to £100K. Phase 2: up to £1 million

Sponsors

GlaxoSmithKline, BASF, Sanofi and Abbvie

*All animal studies have been ethically reviewed and carried out in accordance with national regulatory and legal requirements and company policies on the care, welfare and treatment of animals.

References

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