CRACK IT

SANOFI

gsk do more feel better live longer

Challenge 17: Neuratect

Launch Meeting
12 September 2014

abbvie

BASF
The Chemical Company

Alessandra Giarola, PhD
Manager, CNS Safety Pharmacology
GlaxoSmithKline

National Centre for the Replacement, Refinement & Reduction of Animals in Research

Outline

- The challenge
- Current state of the field
- Scientific benefits
- 3Rs benefits
- Why there is a need
- Deliverables
- Nature of In-Kind support

The Challenge

The goal of the Neuratect challenge is to generate physiologically-relevant human stem cell-based model(s) to identify neurotoxicity (neuronal viability/functional impairment) *in vitro*.

Ideally the platform will integrate morphological/structural endpoints with electrophysiological parameters.

The same platform should also ideally address safety pharmacology applications to assess seizure liability, in order to replace the current gold standard - *ex vivo* hippocampal slice assay.

Current state of the field

Neurotoxicity studies, including developmental neurotoxicity studies, are required for new chemicals entities and for CNS drugs in development. CNS drugs also undergo the seizure assessment for safety pharmacology. Regulatory requirements rely largely on animal studies.

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 .

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

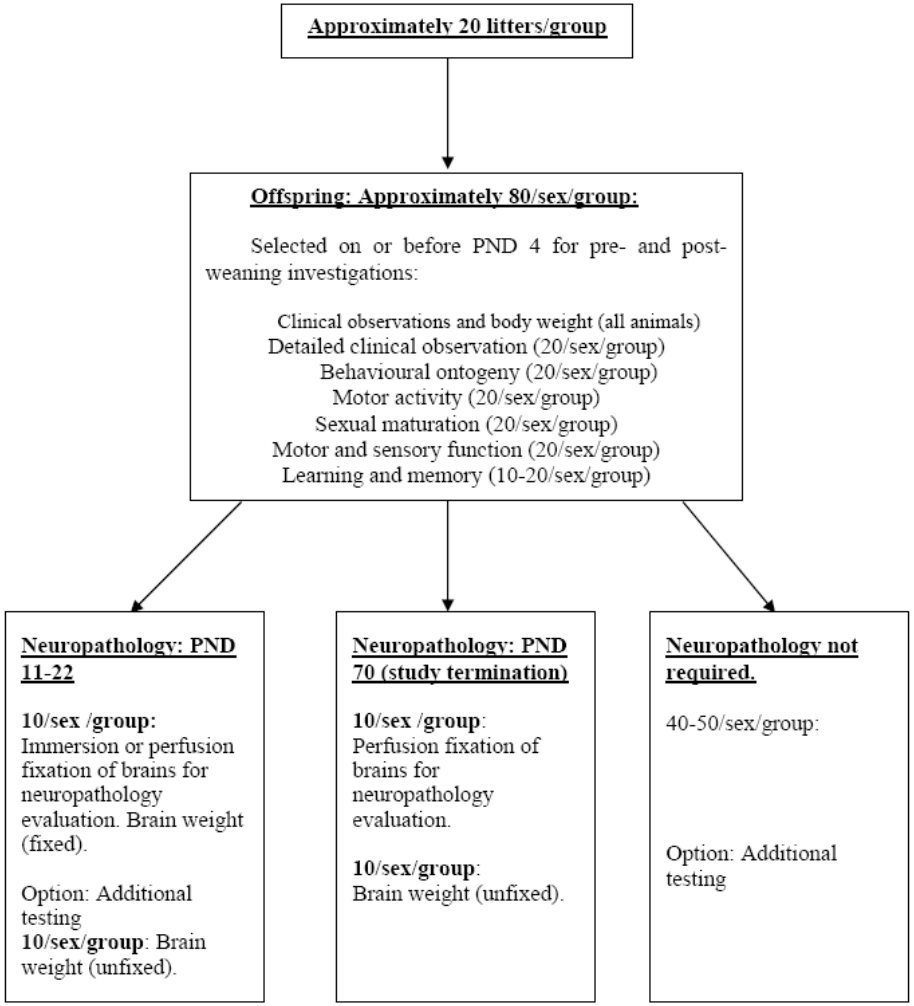
• Neurobehavioural deficits (e.g. Functional Observational Battery (FOB)/Irwin).

• Alterations to learning and memory (e.g. Water maze, T/Y-maze).

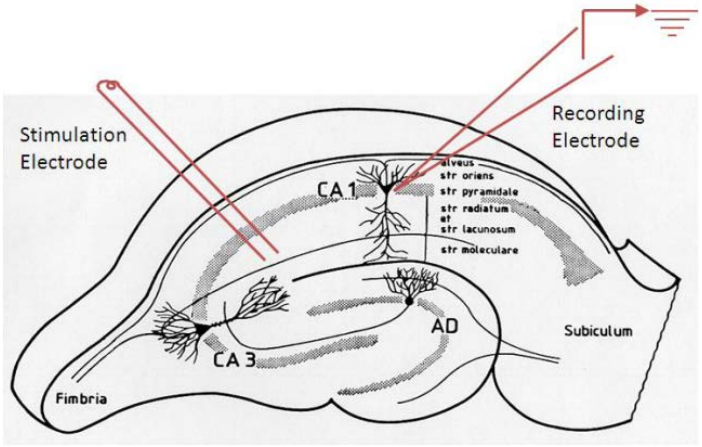
• Loss of sensory and motor function(e.g. startle response, gait analysis, grip strength).

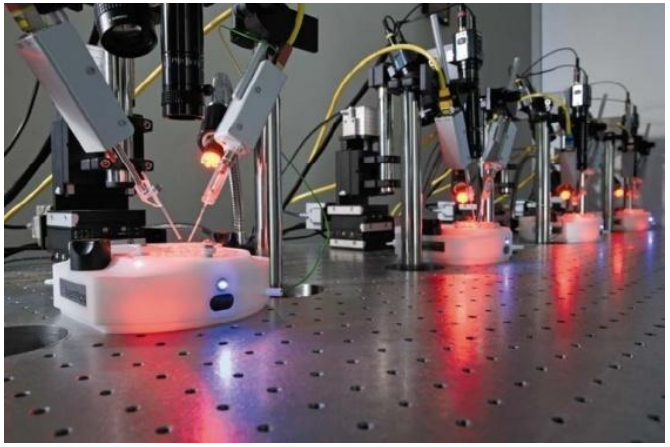
Seizure assessment for safety pharmacology is performed using a stepwise approach starting with ex *vivo* assays followed by *in vivo* models.

General testing scheme for functional/behavioural tests, neuropathology evaluation and brain weights



Ex-vivo hippocampal slice assay





Transverse hippocampal slices from Sprague Dawley/Han Wistar male rats (5-6 week old)

Synaptic activity responses are monitored by placing a bipolar stimulating electrode on the Schaffer collateral pathway and the evoked population spikes (PS) recorded with a microelectrode on the CA1 pyramidal cell layer.

Why there is a need

1. Animal studies are not always predictive of potential adverse effects in humans
2. Large number of animals are used (up to hundreds/study)
3. Time consuming
4. Cost implications (up to £1 million)
5. No high throughput testing of chemicals and pharmaceuticals.

Scientific benefits

Advances in technology and limited utility of animal models, as well as associated welfare concerns, have resulted in scientists addressing more predictive alternatives for toxicological testing of new compounds for neurological toxicity.

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.

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.

• 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.

Deliverables

A successful platform would:

• require the use of small quantities of compound and cells;

• have a high-throughput (between 10 to 20 compounds/week) capability consistent with its use in identifying the potential neurotoxicological effects of large numbers of test substances;

• permit the integration of morphological/structural endpoints with functional (i.e. electrophysiological) endpoints

Phase I

➢ 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

➢ Definition of a set of acceptance criteria for neuronal viability and function based on morphological, structural and electrophysiology readouts.

Phase II

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:

❖ 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 .

Nature of In-Kind support

In-Kind support will be provided by Sponsors

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.

Thank you

The sponsors are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

Contacts are:

Alessandra Giarola – GlaxoSmithKline alessandra.s.giarola@gsk.com

Ludmilla Mazelin - Sanofi ludmilla.Mazelin-Winum@sanofi.com

Thomas Hudzik – Abbvie thomas.hudzik@abbvie.com

Tzutzuy Ramirez – BASF SE tzutzuy.ramirez-hernandez@basf.com

NC3Rs Project Manager

Catherine Vickers – NC3Rs Cathy.Vickers@nc3rs.org.uk

NC 3Rs

CRACK IT

References

OECD (Organisation for Economic Co-operation and Development) (2007), Test guideline 426, Developmental Neurotoxicity Study.

U.S. EPA (U.S. Environmental Protection Agency) EPA/630/R-95/001F 1998, Guidelines for REACH http://www.hse.gov.uk/reach/

ICH S7A Safety Pharmacology Studies For Human Pharmaceuticals (2001). Guidance for Industry. Published in the Federal Register, Vol. 66, No. 135; July 13, 2001 M3

ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, December 2009 EMA/CPMP/ICH/286/1995

Pampaloni *et al*., (2007). The third dimension ridges the gap between cell culture and live tissues. *Nature Rev Molec Cell Biol.* 8: 839-845

Lee e*t al.,* (2009). In vitro toxicity testing of nanoparticles in 3D cell cultures. *Small*. 5: 1213-1221

Buzanska *et al*., (2009). A human stem cell based model for identifying adverse effects of organic and inorganic chemicals on the developing nervous system. *Stem Cells.* 27:10, 2591-2601

Novellino *et al*., (2011). Development of micro-electrode array based tests for neurotoxicity: assessment of interlaboratory reproducibility with neuroactive chemicals. *Frontiers in Neuroengineering*. 4(4): 1-14

Robinette B *et al*., (2011). In-vitro assessment of developmental neurotoxicity: use of microelectrode arrays to measure functional changes in neuronal network ontogeny. *Frontiers in Neuroengineering*, 4(1): 1-9

Ylä-Outinen L *et al*., (2010). Human cell-based micro electrode array platform for studying neurotoxicity. *Frontiers in Neuroengineering*, 3(111): 1-9

Smirnova L *et al*., (2014). Developmental neurotoxicity - challenges in the 21st century and in vitro opportunities. ALTEX. 31(2):129-56.

NC 3Rs

CRACK IT