PROPOSAL:
Translational Safety Biomarker Assessment of Neurotoxicity

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Emerging Issues Session
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Objective

Develop an understanding of the importance of fluid-based biomarkers as translational safety biomarkers used in addition to current assessment tools (histology).

- Attrition and adverse side-effects of drugs in clinical development due to neurotoxicity amount up to 25%
- Comparable to nephro- and hepatotoxicity
- Not restricted to CNS-targeted drugs
Current gaps in the assessment of neurotoxicity

- Lack of sensitivity and quantitative nature of histology (Dosing)
- Spatial restrictions in sampling (8 coronal sections, CNS vs. PNS)
- Invasive nature of sampling and lack of longitudinal measurements
- Lack of translational character from pre-clinical to clinical
Why new safety biomarkers?

- Translational biomarkers for early clinical safety
- Minimally invasive for longitudinal assessment
- Increased understanding of toxicological mechanism
- Target engagement – understanding of toxicity mechanisms
- Earlier decision making and de-risking
- Potential to speed up non-clinical drug safety
- Animal models not always predictive of toxicity in humans
- Current endpoints related to late stage effects
- Drug attrition often related to toxicity/safety
<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Regional Target</th>
<th>Cellular Target</th>
<th>Subcellular Target</th>
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<tbody>
<tr>
<td>TMT</td>
<td>Limbic Structures</td>
<td>Neurons</td>
<td>Perikarya</td>
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<td>MK-801</td>
<td>Cortex</td>
<td>Neurons</td>
<td>Perikarya?</td>
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<td>Kainate</td>
<td>Limbic Structures</td>
<td>Neurons</td>
<td>Perikarya</td>
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<td>MPTP</td>
<td>Neostriatum</td>
<td>Nigral-Striatal Dopamine Neurons</td>
<td>Dopaminergic Nerve Terminals</td>
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<td>Bilirubin</td>
<td>Cerebellum</td>
<td>Purkinje Cells</td>
<td>Perikarya</td>
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<td>Cadmium</td>
<td>Striatum</td>
<td>Neurons, Gila</td>
<td>?</td>
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<tr>
<td>6-OH DA</td>
<td>Neostriatum</td>
<td>Dopamine Neurons</td>
<td>Terminals and Perikarya</td>
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<td>5,7-DHT</td>
<td>Cortex, Hippocampus Striatum</td>
<td>5-HT Neurons</td>
<td>5-HT Nerve Terminals Perikarya</td>
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<tr>
<td>Colchicine</td>
<td>Hippocampus</td>
<td>Dentate Neurons</td>
<td>Perikarya</td>
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<td>3-Acetyl Pyridine</td>
<td>Inferior Olive</td>
<td>Neurons</td>
<td>Perikarya</td>
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<td>Methamphetamine</td>
<td>Neostriatum</td>
<td>Dopamine Neurons</td>
<td>Dopamine Nerve Terminals</td>
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<td>IDPN</td>
<td>Brain Stem</td>
<td>Neurons</td>
<td>Neurofilaments</td>
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After cell death detection is no longer possible, the debris from axon degeneration can still be detected (from Robert Switzer)
Fluid-based biomarker of neurotoxicity

- Increasing body of scientific literature and evidence for fluid-based biomarkers of neurotoxicity
- Immunoassay-based measurements of brain-injury biomarkers
- Development of multiplexed (up to 130 proteins/peptides) validated multiple-reaction monitoring-based assays
Classical Stains Don’t Reveal Subtle Damage

Provided by James O’Callaghan, CDC
The dopaminergic neurotoxicant, MPTP, results in a rapid induction of astrogliosis in the affected region of mouse brain (striatum). (A) Immunohistochemical analysis of GFAP reveals a time-dependent astrogliosis following MPTP (12.5 mg/kg, s.c.). (B, C) Levels of GFAP mRNA (—) and protein (■) were measured in striatum by TaqMan® real-time PCR or sandwich ELISA and are represented as fold or % increase over corresponding saline-treated controls, respectively. (D) GFAP protein levels in various brain regions (CER= cerebellum;CTX= cortex; HIP= hippocampus; HYP= hypothalamus, STR= striatum) of saline (■) or MPTP (■) treated mice, 48h post MPTP.

provided by James O’Callaghan
Biomarkers of neurotoxic mechanisms

Modified after Mondello et al., 2011
The Proposal

Year 1:
- Gap analysis of current neurotoxicity assessment strategies by multidisciplinary neurotoxicity working group
- Literature review and, if accessible, review of internal data of multimodal biomarkers in neurotoxicity
- Assessment of biomarker assays and (commercial) solutions - status of development
- Preparation of agenda for neurotoxicity workshop and nomination of compounds/models
- Preparation of white paper
The Proposal, continued

Year 2:

- Experimental study design of neurotoxicity models and biomarker evaluation: modality/biomarkers to be measured in comparison to histology
- Definition of endpoints