Preclinical Screening for CNS effects of potential drug substances

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Agenda

1. Background
2. In vitro
3. *in vivo* behavioral test - FOB/Modified Irwin
4. Assessment of seizures/convulsions
5. Translational considerations
6. Conclusion
Introduction

• The nervous system (and the human brain in particular) is by far the most complex organ but hardly understood.

Hippocrates (about 400 B.C.)
...all the most acute, most powerful, and most deadly diseases, and those which are most difficult to be understood by the inexperienced, fall upon the brain.

[Diagram of the nervous system showing the central nervous system (CNS) and peripheral nervous system (PNS)]

H. Simpson’s X-ray
# Attrition - serious ADRs - withdrawal

<table>
<thead>
<tr>
<th>Phase</th>
<th>‘Nonclinical’</th>
<th>Phase I</th>
<th>Phase I-III</th>
<th>Phase III/Approval</th>
<th>Post-Approval</th>
<th>Post-Approval</th>
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</thead>
<tbody>
<tr>
<td>Information:</td>
<td>Causes of attrition</td>
<td>Serious ADRs</td>
<td>Causes of attrition</td>
<td>ADRs on label</td>
<td>Serious ADRs</td>
<td>Withdrawal from sale</td>
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<tr>
<td>Sample size:</td>
<td>88 CDs stopped</td>
<td>1,015 subjects</td>
<td>82 CDs stopped</td>
<td>1,138 drugs</td>
<td>21,298 patients</td>
<td>47 drugs</td>
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<td>Cardiovascular:</td>
<td>27%</td>
<td>9%</td>
<td>21%</td>
<td>36%</td>
<td>15%</td>
<td>45%</td>
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<tr>
<td>Hepatotoxicity:</td>
<td>8%</td>
<td>7%</td>
<td>21%</td>
<td>13%</td>
<td>0%</td>
<td>32%</td>
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<tr>
<td>Haematology/BM:</td>
<td>7%</td>
<td>2%</td>
<td>4%</td>
<td>16%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td>Nervous system:</td>
<td>14%</td>
<td>28%</td>
<td>21%</td>
<td>67%</td>
<td>39%</td>
<td>2%</td>
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<tr>
<td>Immunotox; photosensitivity:</td>
<td>7%</td>
<td>16%</td>
<td>11%</td>
<td>25%</td>
<td>34%</td>
<td>2%</td>
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<tr>
<td>Gastrointestinal:</td>
<td>3%</td>
<td>23%</td>
<td>5%</td>
<td>67%</td>
<td>14%</td>
<td>2%</td>
</tr>
<tr>
<td>Reprotox:</td>
<td>13%</td>
<td>0%</td>
<td>1%</td>
<td>10%</td>
<td>0%</td>
<td>2%</td>
</tr>
<tr>
<td>Musculoskeletal:</td>
<td>4%</td>
<td>0%</td>
<td>1%</td>
<td>28%</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Respiratory:</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>32%</td>
<td>8%</td>
<td>2%</td>
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<tr>
<td>Renal:</td>
<td>2%</td>
<td>0%</td>
<td>9%</td>
<td>19%</td>
<td>2%</td>
<td>0%</td>
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<td>Genetic tox:</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Carcinogenicity:</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Other:</td>
<td>0%</td>
<td>0%</td>
<td>4%</td>
<td>16%</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

With permission of JP Valentin, AZ. Adapted from Redfern WS et al. SOT 2010 Poster 1081
The context: CNS side effects during drug development

- In principle CNS side effects can be caused by all classes of drugs. However, a higher frequency is expected for molecules produced to target CNS tissue.

- Understanding of CNS side effects can be challenging due to the following reasons:
  - The relative lack of knowledge of fundamental biology and pathophysiological underpinnings of many CNS disorders
  - The relatively poor predictive validity of preclinical models, and lack of accepted biomarkers
  - The relatively high use of subjective investigator and patient-rated diagnostic scales resulting in heightened placebo response
  - The relatively novel mechanism of action for many new CNS drugs

- For most CNS disorders small molecule approaches are used (exposure in target compartment) which usually show greater side-effect profiles in comparison to biotherapeutic approaches.

- Many CNS drugs are metabolized by CYP 3A4 or 2D6 pathways increasing the drug’s risk-to-benefit ratio via potential drug-drug interactions.
Neurotoxicity has many flavors:

- Neurotoxicity is a form of toxicity in which a biological, chemical, or physical agent produces an adverse effect on the structure or function of the central and/or peripheral nervous system.
  - Neuronal cell death/cytotoxicity
  - Behavioral changes/mood disorders
  - Seizures/convulsions
  - Drug dependence and abuse
  - Suicidal ideation
  - other
Important questions

• Does the compound enter the brain?
• Is there a centrally mediated mechanism (although non-CNS indication)?
• Does the target control additional mechanisms/pathways?
• Is there CNS relevant off-target activity?
### Off-target receptor screen is an important pillar in early drug development and safety

<table>
<thead>
<tr>
<th>CNS receptor/target</th>
<th>Associated neurological/psychoactive effects (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1</td>
<td>Agonism: alteration of cognition and memory, sleep disturbance, drowsiness, sedation, locomotor dysfunction. Antagonism: emesis, depression, <strong>suicidal tendencies</strong>.</td>
</tr>
<tr>
<td>D1</td>
<td>Agonism: may induce dyskinesia, extreme arousal, flushing, nausea, vomiting, dizziness, locomotor activation. Antagonism: tremor, sedation, depression, anxiety and <strong>suicidal intent</strong> (D1 and D5 antagonists).</td>
</tr>
<tr>
<td>D2</td>
<td>Agonism: drowsiness, dizziness and nausea, psychosis after long term treatment; (Aripiprazole received a box warning for <strong>suicidal ideation</strong>). Antagonism: tardive dyskinesia (impairment of voluntary movement), akathisia (an inability to sit still or remain motionless) and other extrapyramidal effects (Parkinson-like syndrome).</td>
</tr>
<tr>
<td>D3</td>
<td>Agonism: cognitive and emotional functions, psychosis, neurodegeneration, coordination, substance abuse, sedation, schizophrenia. Antagonism: indistinguishable from the side effects given for D2 antagonists.</td>
</tr>
<tr>
<td>D5</td>
<td>Agonism: no relevant CNS side effects known. Antagonism: <strong>suicidal intent</strong> (observed with D1 and D5 antagonists)</td>
</tr>
<tr>
<td>DAT</td>
<td>Activators: can stimulate dopamine uptake (amphetamine-like drugs) Inhibitors: prevent dopamine uptake (cocaine-like drugs), highly addictive psychostimulants, important effects on locomotor activity, motivation, reward and cognition, dopaminergic hyperactivity, ADHD, depression, Parkinsonism, psychotic disorders, seizure, dystonia, dyskinesia.</td>
</tr>
<tr>
<td>GABA-A</td>
<td>Agonism: inhibition of most neuronal systems, strongly interfering with the function of the brain, causing sedation, movement disturbances, hallucinations and potentially leading to tolerance. Antagonism: mimics the symptoms of epilepsy, potential anxiogensics and proconvulsivants.</td>
</tr>
</tbody>
</table>

Examples of off-targets associated with neurotoxicity
Receptor binding: Principle of Receptor HT assays

Carefully designed panel of human target-based assays (80+) with known links to clinical adverse effects
- 1 or 2 references are tested in each assay to validate the experiments
- turn around time ~ 15 days
- Current capacity : ~ 1500 compounds / year
Data interpretation: Comparison to exposure levels and adverse events of marketed drugs

Gene symbol: GABRA1
Entrez Gene ID: 2554
Class/Species: Ion Channel/Homo sapiens

Clinical and Marketed Drug Information (TOP 5)

<table>
<thead>
<tr>
<th>Therap. use</th>
<th>Midazolam hydrochloride</th>
<th>Clobazam</th>
<th>Alprozolam</th>
<th>Temazepam</th>
<th>Oxazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthesia</td>
<td>0.002</td>
<td>0.300</td>
<td>0.004</td>
<td>0.044</td>
<td>0.030</td>
</tr>
<tr>
<td>Epileptic seizure</td>
<td>0.030</td>
<td>2.215</td>
<td>0.024</td>
<td>0.115</td>
<td>0.056</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NA</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Major Adverse Drug Reactions (Organized by System Organ Class)

- NERVOUS SYSTEM DISORDERS: SOMNOLENCE, DIZZINESS, SEDATION, AMNESIA, MOTOR DYSFUNCTION
- PSYCHIATRIC DISORDERS: DEPRESSION, DRUG ABUSE

A TARGET SAFETY MARGIN of 10 means that the clinical free plasma exposure of your molecule should be at least 10 times below its IC50 on the target to minimize the risk that the target-related ADRs appear in the clinic. An estimated human exposure providing a compound safety margin below 10 increases the risk to see the target-related ADRs developing in man.
Receptor binding:

Points to consider:

- A receptor panel is a powerful tool to get an early overview on potential safety liabilities which can help to design out certain toxicophores

- Interpretation should be based on the effective clinical concentration of the desired drug.

- The relevance of a potent receptor modulation should be followed up by an appropriate next level assay (functional proof).

- Profiling vs competitor compound or reference compounds on the market

- Useful to built in silico models

- Knowledge on the effects of CNS off-targets is a requirement for a drug-induced abuse/dependence as well as for a prospective suicidality assessment

- Somebody’s target is somebody else’s off-target

<table>
<thead>
<tr>
<th>Assay</th>
<th>IC_{50}</th>
<th>Assay</th>
<th>IC_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT1A</td>
<td>&gt; 10</td>
<td>h Motilin</td>
<td>9.7</td>
</tr>
<tr>
<td>5HT2A</td>
<td>12.7</td>
<td>M1</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>5HT2C</td>
<td>&gt; 10</td>
<td>M3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Ad1</td>
<td>&gt; 30</td>
<td>op-delta</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Ad2A</td>
<td>&gt; 30</td>
<td>op-kappa</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Ad3</td>
<td>&gt; 30</td>
<td>op-mu</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Alpha1A</td>
<td>&gt; 10</td>
<td>TP</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>alpha2B</td>
<td>&gt; 10</td>
<td>Y1</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>alpha2C</td>
<td>&gt; 10</td>
<td>hr V1a</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>beta1</td>
<td>&gt; 10</td>
<td>hr V2</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>beta2</td>
<td>&gt; 10</td>
<td>r BzD</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>AT1</td>
<td>&gt; 30</td>
<td>r GABA A</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>B2</td>
<td>&gt; 30</td>
<td>Nic(ns)</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>CB1</td>
<td>&gt; 10</td>
<td>r PCP</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>CCKa</td>
<td>&gt; 30</td>
<td>5HT3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>CCKb</td>
<td>&gt; 30</td>
<td>r Ca2+(L)</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>D2</td>
<td>&gt; 10</td>
<td>AR</td>
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<td>ERα</td>
<td>&gt; 30</td>
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<tr>
<td>GHS</td>
<td>&gt; 30</td>
<td>PR+R</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>H1</td>
<td>&gt; 10</td>
<td>hr PXR</td>
<td>&gt; 30</td>
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<tr>
<td>H3</td>
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<td>OR</td>
<td>&gt; 30</td>
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<td>AdT</td>
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<td>COX-2</td>
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<tr>
<td>MAO-A</td>
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<tr>
<td>h PDE3</td>
<td>&gt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h PDE4D</td>
<td>&gt; 10</td>
<td></td>
<td></td>
</tr>
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</table>
Core Battery: Central Nervous System

In vivo studies:

Effects of a test substance on the CNS should be assessed appropriately:

- Behavioral changes
- Coordination
- Motor activity
- Sensory/motor reflex responses
- Body temperature

Use of a modified Irwin’s test (Irwin, 1968), a Functional observation battery (FOB) (Mattson et al., 1996), or other appropriate test (Haggerty, 1991)
CNS Core battery tests

Modified Irwin test

Parameters (more than 50 observations)

Behavior
➢ Arousal, grooming, handling reactivity, vocalization, stereotypic/ bizarre behavior

Motor activity and coordination
➢ Body tone, body and tail position, spontaneous locomotor activity, rearing, gait, motor coordination, paralysis

Autonomic profile
➢ Lacrimation, salivation, pupil size + reflex, palpebral closure, skin color, urination, defecation, piloerection, exophthalmos, respiration, effects on body temperature

Sensory/motor reflex responses
➢ Approach, tail pinch response, startle response, visual placing, reflexes (righting, corneal, pinna, flexion)

It is a regulatory requirement: According to ICHS7A this test needs to be done prior to first in human studies
**CNS Core battery tests**

*Modified Irwin test*

**Principle:**
- Pre- and post dose of single (oral) administration in the rat
- Treatment groups: vehicle, test item (dose range), reference item (optional)
- Requires experienced observer, blinded to treatment allocation
- Assessments performed from least (observations) to most (manipulation) stressful
- Observations in the home cage and inside/outside an observation arena
- Scoring: absence/presence, grades
- Measurement of body temperature [°C]
- Test item groups are (statistically) compared to the vehicle-treated group
**CNS Core battery tests**

*Modified Irwin test: Diazepam*

Positive modulator of GABA-A receptors

↑ inhibitory neurotransmission

Effects in humans:

- Hypnotic, sedation, anxiolytic
- Muscle relaxation

(n=4/group)

**Arousal**

- Vehicle
- Diazepam 3 mg/kg
- Diazepam 30 mg/kg
- Diazepam 60 mg/kg

**Tail position**

- Vehicle
- Diazepam 3 mg/kg
- Diazepam 30 mg/kg
- Diazepam 60 mg/kg

**Body posture**

- Vehicle
- Diazepam 3 mg/kg
- Diazepam 30 mg/kg
- Diazepam 60 mg/kg
Assessment of seizurogenic effects

Background

• Seizure ≠ Convulsion

• Seizure:
  – result of spontaneous excessive neuronal discharges in the CNS
  – The type of seizure depends on where in the brain the electrical impulse originates. Different types of seizures have different symptoms, including convulsions
  – Not all seizures produce convulsions (e.g. absence seizures)
  – potentially seen in all animal species (with dogs often being most sensitive) and all therapeutic areas

• Convulsion:
  – body muscles contract and relax rapidly and repeatedly, resulting in an uncontrolled body shaking
  – Not all convulsions are produced by seizures
    – fever, hypoglycemia, meningitis, stroke, uremia, head or brain injury and withdrawal from sedatives
Why do people seize?

• Impaired inhibition:
  – e.g. GABA_A antagonism or GABA_B agonism
  – Adenosin antagonism

• Enhanced excitation:
  – NMDA (glutamate receptor) and other excitatory amino acids

• Disordered conduction
  – Neuronal sodium channel blockade

• Indirect causes
  – Metabolic failure, Oxigen, glucose, sodium, etc.
  – Pathological alterations (e.g. tumor, change of white matter)
Non-clinical seizure assessment

Test options:

• Binding
  – Pharmacological profiling against the relevant off-targets may provide the first indication of a potential seizure liability

• In vitro electrophysiology
  – Patch clamping of relevant ion channels (e.g. GABA or neuronal sodium channels, NMDA, etc.)
  – Electrophysiology with primary rat neurons or neuronal networks from human stem cells using multi-electrode arrays
  – in vitro slice preparations (e.g. rat hippocampus, see next slide)

• Observations in CNS Safety Pharmacology or Toxicity studies
  – Signs of CNS excitability: tremors, twitches, convulsions

• Pro-convulsant models in rodents
  – Chemically induced (PTZ, Picrotoxin)
  – Electrically induced (Maximal Electroshock Seizure (MES) test)
  – Seizure prone animals (e.g. audiogenic seizures in BDA/2 mice, GAERS or Wag/Rij rats)
  – Epileptic animals (kindling, either electrically or chemically)

• EEG recordings (gold standard)
  – Various species, stand alone or integrated in a toxicity study
  – Provides the most sensitive assessment \(\rightarrow\) epileptiform abnormalities

Derisking requires the most appropriate test(s) in the most sensitive species
In vitro test systems: rat hippocampal slice preparation (Easter et al., JPET, 2007)

- Able to detect direct effects of a wide range of compounds associated with seizure induction in man
  - Endpoint: frequency and AUC of population spikes measured with electrodes
- Hippocampus is strongly linked to partial seizures, incl temporal lobe epilepsy

- Limitations: one part of the brain, no chronic exposure, no BBB, indirect effects not addressed
In vivo test systems: Zebra Fish (Dario rerio) (Winter et.al. JPTM, 2008)

- Assessment of movement pattern in 7d old Zebrafish larvae using a videotracking system
- 25 reference cpds were tested at 5 concentrations for 1 h
- seizure/convulsive like locomotory patterns: dramatically increased swimming speed/activity (named stage I); rapid “whirlpool” motion circular swimming (stage II); loss of posture and loss of motion for 1–3 s (stage III)
- Predictivity of the convulsant assay: 72%, consisting of a positive control predictivity rate of 77% and a negative control prediction rate of 63%.
  - Regarded as a suitable medium throughput, early in vivo screen
  - Limitations: fish specific metabolism, absorption/bioavailability administration

Movement pattern in 24 well plate after PTZ application
In vivo test systems: standard safety/tox studies

• Observation in core battery CNS test (rat; Mod. Irwin test, FOB)
  – tremors, twitches, convulsions

• Convulsions can be observed in repeated dose tox studies but need to be differentiated from tremors, muscle twiching, etc, which can also have peripheral reasons (need of trained personal)
  – It is important to know the spontaneous seizure/convulsion rate of your test animal/strain

• In comparison to specific seizure studies the sensitivity is low
  – assessment at fixed time points
  – Difficult to distinguish from general behavior changes
  – Single administration (non-convulsive dose may induce convulsion upon multiple dosing „chemical kindling“)
Pro-convulsant activity
Pentylenetetrazole (PTZ) Test

• Purpose
  – detection of pro-convulsant (and/or anti-convulsant) activity

• Principle
  – PTZ is a GABA-A receptor antagonist
  – Different designs possible:
    – Threshold dose of PTZ + different doses of test drug: increase in No. of convulsing animals?
    – Timed i.v. infusion of PTZ (preferred option): latency to different convolution stages
      – Sensitive to pro and anti-convulsant effects

• Reference compounds examples:
  – Pro-convulsant: FG7142
  – Anticonvulsant: diazepam

From: Löscher, Eur J Pharmacol 21, 2009
In vivo EEG measurements

- Seizure usually preceded by sharp waves and followed by low frequency rhythmic activity (rat)

Seizure associated abnormalities almost always involved increases in spike amplitude

PTZ-induced seizure in the rat (Nishida et al., Experimental Neurology, 2007)
Translational considerations
Assessing the predictive value of the rodent neurofunctional assessment for commonly reported adverse events in phase I clinical trials


CNS-related safety data on 141 small molecule from five pharmaceutical companies were analyzed to identify the concordance between rodent multiparameter neurofunctional assessments (Functional Observational Battery and the five most common adverse events (AEs) in Phase I clinical trials, namely headache, nausea, dizziness, fatigue/somnolence and pain.
Analysis Methods:

Non-clinical and clinical data on small molecules that generated side effects in Phase I clinical trials were shared by questionnaire and anonymized.

- Compounds which progressed to FIH testing between 2000 and 2011 were selected (n=141)

-Predictive value of the FOB or Irwin study for clinical adverse events was assessed at equivalent mean free plasma drug exposure (free Cmax). This assessment was carried out at one, three, 10 and 30 times mean clinical Cmax (where no effect was observed) or the lowest clinical exposure level (where an effect was observed).

- Two analyses were carried out
  - Receiver Operator Characteristics (ROC) curves:
    1) Overt Toxicity analysis: comparison of any evidence of non-clinical AE with any of the selected clinical AE
    2) Plausible Correlate analysis: comparisons between plausibly related non-clinical and clinical AEs
Incidence of clinical AES and plausible non-clinical correlates

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>Preclinical plausible correlate (rodent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>[None]</td>
</tr>
<tr>
<td>Nausea</td>
<td>↓ body weight gain OR ↓ food consumption</td>
</tr>
<tr>
<td>Dizziness</td>
<td>↓ rearing OR ↓ LMA horizontal activity OR ↓ LMA rearing</td>
</tr>
<tr>
<td>Somnolence/ Fatigue</td>
<td>↓ home cage arousal OR hunched posture OR ↓ grip strength / traction response OR ↓ handling reactivity/aggressiveness OR ↓ rearing OR ↓ LMA horizontal activity OR ↓ LMA rearing</td>
</tr>
<tr>
<td>Pain</td>
<td>vocalisation OR ↓ rearing OR ↓ LMA horizontal activity OR ↓ LMA rearing</td>
</tr>
</tbody>
</table>
**Results:**

ROC plots for the clinical AEs across the four exposure multiples examined.

Sensitivity: rate of true positives
Specificity: rate of true negatives
1-specificity: false positive rate

Dashed line indicates line of unity, test performance with no value
Conclusion of neurofunctional assessment for clinical phase 1 studies

- Specific CNS endpoints in the rodent neurofunctional assessment neither predict nor detect the most commonly observed specific CNS related AE’s in the FIH study (plausible associations analysis).

- The presence of CNS findings non-clinically does not predict the presence of any of the 4 clinical AEs analyzed (overt toxicity analysis).

- This raises the question as to whether the rodent neurofunctional assessment can be used to reliably predict the occurrence of the most commonly observed spontaneously reported subjective CNS-related AE’s in the FIH study.
Predictive value of animal findings for human toxicities

Correlations depend on animal species and organ systems

Table 4. Comparison of target organs and correlation of adverse drug reactions between small and large molecule drugs

<table>
<thead>
<tr>
<th>Target organ of ADRs</th>
<th>Small molecule drugs</th>
<th>Large molecule drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of ADRs</td>
<td>% of correlation</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td>Neurological</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>Hepatobiliary</td>
<td>11</td>
<td>73</td>
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<tr>
<td>Hematological</td>
<td>8</td>
<td>75</td>
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<tr>
<td>Cutaneous</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>Systemic</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>Ocular</td>
<td>5</td>
<td>64</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Metabolic</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>Faecal/oral</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>Urinary</td>
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<td>61</td>
</tr>
<tr>
<td>Respiratory</td>
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<td>45</td>
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<tr>
<td>Infection</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Nasal</td>
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<td>27</td>
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<tr>
<td>Application site reactions</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>45</td>
</tr>
</tbody>
</table>

Abbreviation: ADRs, adverse drug reactions.
Percentage of correlation was calculated for each ADR by dividing sum of drugs categorized into a to d by a total number of drugs reporting the ADR.


Conclusion

• The non-clinical prediction of CNS related adverse events «neurotoxicities» is very challenging
• The translation value of non-clinical assays can be improved and is for some assays not thoroughly assessed
• Combination of different assays increases predictivity (there is not «the one» assay who tells all)

→ There is definitively the need for more modified/alternative test systems with increased predictive power
  - Human iPS cells?
  - Cerebral organoids
  - Animal disease models?
  - ?

• Identification and validation of more translational (ideally fluid biomarkers) such as microRNAs, F₂-isoprostanes, translocator protein, glial fibrillary acidic protein, ubiquitin C-terminal hydrolase L1, myelin basic protein, microtubule-associated protein-2
Reintroducing neuronal complexity... the future?


Complex morphology but recapitulates various human brain region including cortex like structures, fore-, mid-, hindbrain and Hippocampus, cavities reminiscent of brain ventricles.
CNS safety models – what are the options?

“All models are wrong, but some are useful”

George E. Pelham-Box, October 18, 1919 – March 28, 2013. British mathematician and Professor of Statistics at the University of Wisconsin

It is essential to interpret data within the known and predefined limits of the model.
Thank you
Acknowledgments

- Gregory Friedrichs
- Berengere Dumotier
- Laszlo Urban
- Kurt Zimmermann
- Valerie Weber
- Everybody I forgot to mention
Backups
Pharmacological targets with a positive association to drug-induced seizure

Seizures (+- convulsions) are the result of spontaneous excessive neuronal discharges in the CNS and are a severe safety issue

- G-protein-coupled receptors
  - 5-HT$_{1A-B}$
  - 5-HT$_{2A}$
  - 5-HT$_{2C}$
  - 5-HT$_{6}$
  - 5-HT$_{7}$
  - Adrenergic $\alpha_{1A, 2A-C}$
  - Adrenergic $\beta_{1,3}$
  - Cannabinoid CB$_{1}$
  - Dopamine $D_{1}$
  - Dopamine $D_{2, 3, 4, 4}$
  - Growth hormone secretagogue
  - Histamine $H_{1,2}$
  - Melanocortin $M_{1}$
  - Muscarinic acetylcholine $M_{1}$
  - Muscarinic acetylcholine $M_{2-5}$
  - Opioid $\delta$
  - Opioid $\kappa$

- Ligand-gated ion channels
  - 5-HT$_{3}$
  - AMPA
  - GABA$_{A_{V}}$, agonist site
  - GABA$_{A}$, Flunitrazepam site
  - Glycine, strychnine site
  - Kainate
  - Nicotinic acetylcholine
  - NMDA, agonist site
  - NMDA, glycine site
  - NMDA, phenytoin site

- Voltage-gated ion channels
  - K$_{ATP}$ potassium channel
  - Sodium Channel Site 2
  - L-type calcium channel diltiazem site
  - L-type calcium channel verapamil site
  - K$_{A}$ potassium channel
  - KNCQ2/3 potassium channel

Pentylenetetrazol (PTZ): circulatory and respiratory stimulant, GABA antagonist, induces seizures and convulsions