

Any level of suffering is obviously a concern for everyone, but tackling severe suffering should be a top priority.

Dr Penny Hawkins, RSPCA



RSPCA meeting: Focus on severe suffering - refining severe disease models and procedures

Applying the Roadmap in practice – examples from Novo Nordisk

Karolinska Institutet 24-25 August 2022

Thomas Bertelsen
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Applying the roadmap at Novo Nordisk

Three examples

- 1) Lysosomal Storage Disease (LSD) model – knockout mouse
- 2) Transient Middle Cerebral Artery Occlusion model (tMCAO)
- 3) Maximum Tolerated Dose (MTD)

Why the roadmap works

Applying the roadmap at Novo Nordisk

Prospectively

- Identify as many sources of harm as possible
 - Related to the (disease) model
 - Related to procedures
 - Related to housing, husbandry and care
- Agree on Humane Endpoints
 - General
 - Model specific
- Agree on procedures for welfare assessment

Retrospectively

- Assess actual severity
 - Identify when and why severe harm was experienced by the animal
 - Identify if avoidable harm unintentionally occurred
 - Evaluate the effectiveness of the implemented Humane Endpoints
 - Evaluate the effectiveness of how animal welfare was assessed
- Agree on revisions
- Agree on how learnings are captured and communicated to all relevant people

"LSD KO" mouse

- Lacks a hydrolytic enzyme leading to accumulation of undegraded substrate in the lysosome.
- Affected animals show systemic disease manifestations such as organomegaly and progressive onset of skeletal and neurological disease by three months of age and die by 60-70 weeks of age.

The benefit of LSD KO mouse

Scientific

- The model fully recapitulates the pathophysiology of a severe rare monogenetic human disease. (high degree of translatability)
- Currently with limited treatment options
- Enables the assessment of the efficacy of existing and novel therapeutic avenues

The benefit of LSD KO mouse

Animal welfare

- The progressive onset of the disease requires a dynamic model of animal care and ensures that disease manifestations are constantly monitored to allow for specific adjustments in the animal's environment and handling to minimise suffering

LSD KO mouse

What did the model look like before applying the roadmap

- The degree of suffering was unclear throughout the life history during disease progression
- A generic understanding of pathophysiological manifestations mainly focused on generic humane endpoints

LSD KO mouse

What we did

- Recognize individual variations with regards to the severity and onset of disease symptoms
 - For example, the literature indicates “hind limb tibio-tarsal joint deformity resulting in ankylosis was observed in 6%, 25% and 37.5% of the LSD-KO mice at 3, 6 and 12 months, respectively.”
- Identify non-invasive biomarkers, e.g. urine levels of accumulated substrate, as prognostic tool to predict onset of severe disease manifestations
- More emphasis on biochemical assay readouts that indicate efficacious treatment prior to onset of severe disease phenotype, i.e. use younger, relatively healthier LSD KO
 - this narrowed and optimized the therapeutic/scientifically relevant window

LSD KO mouse

What did the model look like after applying the roadmap

Overcome obstacles

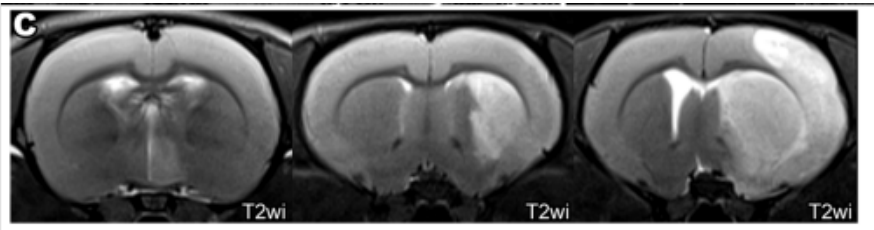
- Intensified monitoring (assisted by biomarkers)
- Euthanasia when Humane Endpoints are reached
- General HEs
- Model specific HEs (related to disease phenotype – e.g. motility)
- Compound specific HEs per protocol
- Intrinsic harm in the housing conditions are addressed
- Non-aversive handling to the extent possible
- Blood sampling – only what is scientifically required (number and volumes)
- Dosing – training and habituation to the extent possible

LSD KO life history

- **At birth:** Male LSD-KO mice are indistinguishable from wild-type and female carrier littermates at birth
- **Week 4:** significant **increases in liver weight** were seen in the LSD-KO mice compared with WT littermates
- **Week 10:**
 - LSD-KO mice begin to exhibit coarse fur and sporadic alopecia, as well as **gibbous deformities** in their hind limb articulations that impede joint mobility
 - the digits of LSD-KO mice appeared thickened when compared with wild-type littermates, and were often found in a **curved, or “claw” position** similar to that often seen in the human disease
- **From week 12:**
 - A hind limb tibio-tarsal joint deformity resulting in ankylosis was observed in 6%, 25% and 37.5% of the LSD-KO mice at 3, 6 and 12 months, respectively
- **Week 32:** all affected mice had developed broadened snouts as a result of abnormal skull development
- **Week 40:** LSD-KO mice demonstrate a noticeable decline in activity; slightly smaller, lower body weight
- **Week 52:** LSD-KO mice were often found moribund in their cages. **Lifespan of the LSD-KO mice is reduced** considerably compared with about 120 weeks in the wild-type mice.

Transient Middle Cerebral Artery Occlusion model (tMCAO)

- The tMCAO model is a stroke model in which a unilateral, non-hemorrhagic focal ischemia-reperfusion injury is surgically induced in rats
- The model has a high construct validity as the vast majority of patients that suffers an ischemic stroke is caused by an transient occlusion of the MCA.
- The drawback is the severity of the model and the variability in lesion size.



T2-weighted MR images of the brain of rats with different stroke sizes (bright areas) 24h after transient middle cerebral artery occlusion. *From Gubskiy, I.L., Namestnikova, D.D., Cherkashova, E.A. et al. MRI Guiding of the Middle Cerebral Artery Occlusion in Rats Aimed to Improve Stroke Modeling. Transl. Stroke Res. 9, 417–425 (2018)*

tMCAO – highlighting refinements (body weight)

- Humane Endpoint – body weight (BW) **before** modification

- Body weight (BW) loss up to 30% compared to the initial bodyweight at start of study
- This high BW loss was accepted because the CRO had experienced that the BW loss was transient and an inherent part of the model

- Humane Endpoint – BW **after** modification

- BW loss up to 20%, corrected for the expected bodyweight gain of the animal during the study

tMCAO – highlighting refinements (body weight)

Impact on animal welfare, assuming that the degree of BW loss reflects the degree of severity:

- Before modification (historical data): **42%** of all animals had a body weight loss of > 20%
- After modification: **29%** of all animals had a body weight loss of > 20% and no animals continued in the study after reaching the 20%

tMCAO – highlighting refinements (post-op care)

- Increased post-operative care to mitigate the initial transient bodyweight loss
 - Fluid therapy to all animals after lesion every time they were handled for other purposes (nine times during the first three days after induction of the lesion)
 - Optimized analgesia regimen (additional buprenorphine doses)
 - Softened food provided in the bottom of the cage twice daily for three days
 - Syringe-feeding if the animal had lost more than 7% of BW

tMCAO – highlighting refinements (seizures)

- Humane endpoints **before** modification

- Epileptiform seizure – one single short seizure fulfils the criterion for euthanasia
- Low-grade seizures were not described

- Humane endpoints **after** modification

- Increased post-op care led to more observations of short (<5 sec) episodes of low-grade focal seizures that were not considered to impact overall animal welfare. An example could be a few seconds of head nodding before the animal continue with normal behaviour
- Consequently, we decided that one short low-grade focal seizure led to increased observation and that more than one additional low-grade focal seizure led to euthanasia
- In addition, one seizure lasting more than 5 sec or a severe seizure (i.e. tonic posturing) led to immediate euthanasia

- Impact on animal welfare

- Better description of model-specific humane endpoints
- Low-grade seizures acknowledged and triggered action (increased observation, and reoccurring low-grade seizures led to euthanasia)

tMCAO – overall conclusion

- A close and constructive dialogue between Novo Nordisk AWB, the Novo Nordisk scientists and the CRO led to:
 - A refined Humane Endpoint on body weight loss
 - A refined Humane Endpoint on seizure episodes
 - A refined post-operative care
- Which has been implemented as a new standard at the CRO

Maximum Tolerated Dose - description of the model

- Study aim: To assess which doses are tolerable and can be used in subsequent regulatory animal studies
- The benefit of the MTD
 - Scientific:
 - Identify a dose level, which can demonstrate organ toxicity in the following pivotal tox studies
 - Identify possible side effects at high dose levels, which may be human relevant
 - Closing of projects early if severe toxicity of expected human relevance is observed
 - Animal welfare: de-risking 'severe suffering' in subsequent regulatory animal studies where many animals are used
 - Rodent studies: up to 264 animals (mouse study)
 - Non-rodent studies: up to 24-40 animals

Maximum Tolerated Dose

To what extent does severe suffering occur?

- defining the level of tolerability unfortunately means also to know when the drug is intolerable
- few animals (rodents max 12; non-rodents maximum 2-4) are expected to experience severe suffering

Maximum Tolerated Dose – our aim to reduce severe suffering

- Ensure that as few animal as possible are subjected to an intolerable dose and that the duration for this is as short as possible
 - Current knowledge about the drug's physical and chemical properties, its potency and mode of action has been investigated in non-animal methods prior to the studies, and this knowledge has been incorporated in the design of the study.
- Groupwise dose-escalation. Depending on the half-life of the drug, the dosing of the next group is not initiated before the tolerability of the lower dose has been evaluated, And this may vary from a single day to several weeks, ensuring that as few animals as possible will be exposed to intolerable doses.

Maximum Tolerated Dose - what did we do

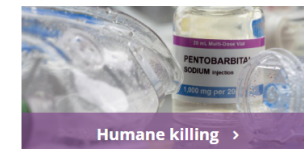
Minimising the number of animals:

- Typical group size in a rodent DRF/MTD study is maximum 6 males and 6 females with one vehicle group and 3 dose groups treated with the test compound
- Typical group size in a non-rodent MTD study is 1 male and 1 female i.e. one group treated with escalating dose levels until the maximum tolerable dose is reached followed by one group of 1 male and 1 female dosed with an expected tolerable dose (without a dose escalating phase)

Maximum Tolerated Dose - what did the model look like after applying the roadmap

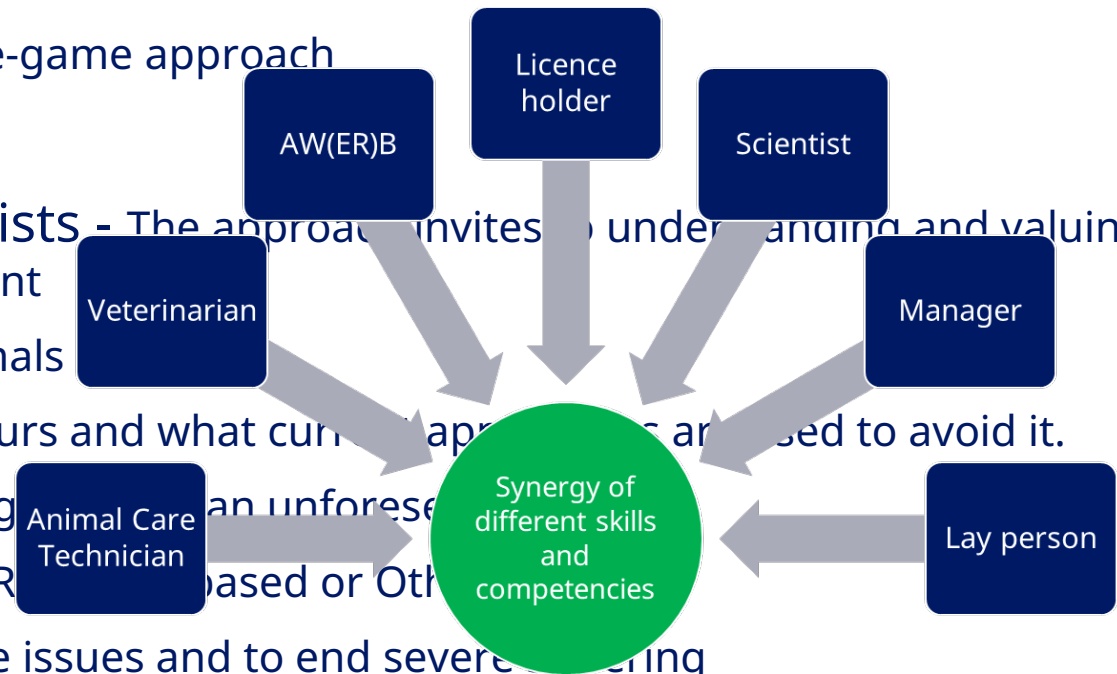
Overcome obstacles

- Intensified monitoring
- Drug holiday
- Stop dosing
- Euthanasia when Humane Endpoints (HEs) are reached
- General HEs
- Compound specific HEs per protocol (e.g. Hypoglycemia, drug related food intake)
- Non-procedure related effects in the housing conditions are addressed
- Non-aversive handling to the extent possible
- Blood sampling – only what is scientifically required (number and volumes)
- Dosing – training and habituation to the extent possible



Why the roadmap works

- The RSPCA approach facilitates a **cooperative response** from licence holders and scientists, because:
 - Objective, data driven, systematic and no blame-game approach
- Dialogue with licence holders and scientists - The approach invites to understanding and valuing the roles of different people within an establishment
 - Data check: Is the scoring as 'severe' for all animals
 - Evaluation: Looking at why severe suffering occurs and what current approaches are used to avoid it.
 - Is the harm prospective or does severe suffering occur as an unforeseen event?
 - Define obstacles: Are the obstacles, - Scientific, Resource based or Other?
 - Overcome obstacles: Set out a plan to overcome issues and to end severe suffering
 - Action plan
 - Evaluate



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Thank you for your attention

- TSBT@novonordisk.com
- Novo Nordisk – the use of animals

Responsible use of animals

We recognise that not all research using animals can be replaced in the foreseeable future and consider it our responsibility to actively support the principles of the 3Rs (Reduce, Refine and Replace research using animals, Professor William Russell and Rex Burch, 1959) internally and externally.



Animal welfare is our

We have a centralised strategic department that contributes to internal awareness and education as well as ensure continued

