Royal Veterinary College
University of London

Summary Minutes: AWERB (PPL review meeting)

Status: Chair approved

Meeting held: 27 April 2021 at 10am via MS Teams

Present
Attendees: 8 plus 1 in attendance and 8 apologies

1 NEW PROJECT LICENCE APPLICATION REVIEW

An application for a project licence to replace an existing project licence had been received. The project licence holder (PPLH) explained that this new licence was a simplified version of the current licence and did not include any new experimental model, species or procedures that were not already in the existing licence.

The PPLH was working on two musculoskeletal disorders: osteoarthritis (OA) and osteoporosis. OA was a disease that caused a lot of pain and there were not many treatments for the pain associated with the disease. The aim of these studies was to contribute to a better understanding of skeletal pain and open up new therapeutic directions for millions of patients affected by OA and fragility fractures.

One of the first objectives was to better understand the mechanisms of pain in osteoarthritis (OA) and how this pain relates to structural and molecular changes in the joint. The team would be examining the contribution of several novel signalling pathways involved in pain in this disease with the aim to identify new molecular targets for the treatment of OA pain.

They would also be working on osteoporosis as it was a major clinical problem and resulted in fractures that caused a lot of pain. They would be examining the contribution of osteoporosis to skeletal pain. They would be using models of bone loss such as ovariectomy and neurectomy to establish the roles of increased osteoclast activity and bone remodelling on bone pain. As osteoporosis occurs in the elderly population, they would also investigate the contribution of ageing to skeletal pain.

Another objective was to establish the mechanisms of pain after fracture. They wanted to better characterise and quantify the pain associated with fracture surgery and healing.

The final objective was the application of biomarkers of pain in preclinical and clinical research. They would be testing the hypothesis that nerve markers related to bone pain could be reproducibly measured in serum of osteoporotic women with pain and that they could be of diagnostic or prognostic value for patients with vertebral fractures.

The following queries were raised by AWERB:

- **Score sheet**: The PPLH had provided a copy of the proposed score sheet, which gave a guide to indicate what should be looked for in each section of the score sheet. The PPLH asked whether it would be better to give a scoring sheet for each of the techniques that were going to be used; or just give a general score sheet and include all the specific adverse effects for each model inside

the licence. The PPLH was advised that the general score sheet should be used throughout the licence, with specific end points for each model.

- **Number of studies planned to do under fracture protocol**: The PPLH had requested up to 500 mice. What was the rationale for that? The PPLH advised that as the project was for 5 years, this would average to using approximately 100 mice per year. It was not expected that all these animals would be required as it was dependent on the outcomes of grant applications that were being submitted. AWERB advised the PPLH against this approach. As there had been significantly less mice used in the current licence, the Home Office Inspector would take that in to account and would want a clear justification of why the numbers were being increased. If the realistic view was that 500 mice were unlikely to be needed, then these numbers should be reduced as the Home Office Inspector would do a harm benefit analysis. If once the study commenced it was found that more numbers were needed, assuming this could be justified, then an amendment could be submitted. It was also noted that ASRU were also encouraging project licence holders to be more realistic about the numbers of animals required and so had designed ASPeL (the system used to apply for project licences) so that it was a straight forward process to apply for an amendment if numbers did need to be adjusted. It was also noted that as part of the mid term project licence review that this would be an opportune moment to review numbers.

- **Analgesia**: AWERB noted that some of the mice would not be receiving analgesia during the post surgical period as pain behaviour was being investigated. They queried that as they were uncomfortable with this. The PPLH advised that for the fracture model, it had already been decided that pain behaviour would not be measured immediately after surgery. Previously pain behaviour had been measured from 3 days, but this was being delayed to 7 days after surgery. This meant analgesia could be provided for up to 1 week after the fracture surgery. This was following advice received on work already done: a paper had been written which explained that in the study mice were only kept up to 6 weeks after fracture and then culled as they were still in pain and that they had started to measure pain behaviour from 3 days after surgery. Some of the reviewers had commented that it was possible there would be not much inflammation immediately after the fracture but that it would probably increase 7 days after surgery. They had therefore recommended waiting 7 days before starting to measure pain behaviour. The project licence would be amended to explain that analgesics would be given for the week after surgery and to explain the reasoning so that it was clear.

- The PPLH advised that the intention was to keep a limited number of mice for up to 12 weeks so the researchers could continue to monitor pain behaviour for a longer period, to see if it went back down to base line (this had not happened within the 6 weeks studies). A query was raised whether going back to base line was clinically relevant? Clinically it was not known if it was relevant because a woman could have an osteoporotic fracture that was undetectable which lasted for a long time. This might not be relevant, but it did need to be checked out, hence why a longer time point was required.

- The PPLH advised that for the longer term study they were looking at the centralisation of pain. For one study, as part of the end points, they collected the dorsal root ganglia to analyse the molecular markers to see if there were any changes as this would be indicative of centralised pain. AWERB advised that as the aim of this study was just to see what would happen (so was really a pilot study), then this should be done on a minimum number of animals. It was suggested that it be done as a separate protocol as it would be a one-off study. The Home Office Inspector would be doing a harm benefit analysis and currently he would be assuming that this would be done to all the animals in the protocol. By indicating it would be a single event with a small number of animals, this would make a difference to the severity category and to the harm

benefit analysis.

An alternative option was to add a section in the animal experience explaining what the majority of the animals would experience but that a subset of less than 10% of the animals would experience being on a longer study for up to 12 weeks.

- A query was raised about the intra-articular injections. It wasn't clear if swollen joints were seen whether the intention was to cull the mice or to keep them. If animals were seen with swollen joints was the assumption that traumatic damage had happened to the cartilage so the animals couldn't be used and needed to be culled? The PPLH explained that sometimes you induced swollen joints, so these animals would not be kept. Intra-articular injections had been done before with no swollen joints so if any were seen this time, they would be re-examined after 24 hours and if still swollen the mice would be culled.

- AWERB pointed out that the project licence mentioned that experiments would use both sex of mice, was that correct though? It was clarified that neurectomy might be done in males, but the majority of the experiments would be in females. AWERB requested that the PPL be amended to stipulate that mainly female mice would be used.

- The project licence mentioned that aged mice might be used in a couple of the protocols. It would be helpful to include any potential adverse effects in relation to the aging mice such as increased incidence of dermatitis and also the potential that the overall animal experience and cumulative effect might be greater if the mice were aged. As there were different definitions for aged mice this would be defined in the project licence as well as including adverse effects to look out for.

- **Protocol one**: It would be helpful to have the variability and effect size values that had been used to calculate power. In particular, how was group size in protocol one decided? The project licence would be amended to make this clearer.

The PPLH was thanked for attending. The project licence would be revised to take into account the comments and suggestions made by AWERB (adverse effects, aged mice, stipulating the numbers). This would then be circulated for another review.

2 MINUTES OF MEETING

The minutes of the meeting held on 8 April 2021 were confirmed as an accurate record of the meeting.

3 ACTION LOG:

3.1 Item 4.2: Breeding and Colony Management discussion group (8 April 2021)

This group had now met. It had been a productive discussion and had promoted general awareness of best practice and suggestions of what could be implemented as well as some action points. It had also provided a reminder of the need to check colonies. There had been a query about archiving which needed to be followed up on as it was not clear how easy it was to get archiving done, what was involved or the costs and putting systems in place to arrange this.

It was agreed that this should be an annual agenda item to review how things were progressing and whether there had been notable changes.

3.2 Item 4.3: NC3Rs PhD studentship scheme (8 April 2021)
Details of this call had been circulated.

3.3 Item 4.4: NC3Rs webinar: refining rodent stereotactic surgeries (8 April 2021)
The webinar recording had been forwarded to researchers who might find this useful. It had mainly focused on general surgery with a spin on stereotactic surgeries but was definitely a good resource.

3.4 Item 7.2 Replacement anatomy pony (8 April 2021)
It had been decided that the potential replacement pony that had been identified, but might be too big, should be trialled to see how he got on. As he wasn't required until the autumn term, it would be arranged for him to arrive in September to give him time to settle in and to get inducted: this would involve students giving him a physical examination practice to see how he tolerated it – this would initially be done in the BSU, so it would be in an environment that he was familiar with; before seeing how he handled being examined in a different location. The training would be done step by step.

3.5 Item 11.1: Virtual AWERB meetings (8 April 2021)
The terms of reference were scheduled to be reviewed at the May meeting.

3.6 Item 5.4: Anatomy pony (12 March 2021)
The farrier had recommended boots rather than shoes. The team would be trained for things they needed to look out for, such as checking for rubbing. The pony would be monitored very carefully when in Camden.

4 BREEDING OF OLDER BITCHES FROM THE DMD COLONY

One of the AWERB attendees declared a potential conflict of interest in this agenda item and offered to leave the meeting but it was agreed that their input would be useful.

Following the discussions held at the previous AWERB meeting, there had been several meetings involving the project licence holder and the technicians about retiring the two older females from the DMD colony. The project licence holder had requested that the females be mated one last time and then retired. AWERB were asked for their views on this, particular in relation to one of the dogs that the technicians had concerns about how they would cope with the breeding process. After discussion it was decided that both dogs should be mated one more time but then rehomed once the pups had been weaned. There did however need to be clear plans going forward so that this situation did not happen again with future dogs. A set of local rules and guidelines needed to be agreed about when female dogs would be automatically retired from the colony. It was also important that the meetings that had recently been set up between the project licence holder and the technicians continued, which would include regular reviews of the dogs so that if there were any concerns about them they were discussed in a timely manner.

5 DATE OF NEXT MEETING

This was scheduled for 11 May at 11am.

Secretary
5 May 2021