Royal Veterinary College
University of London

Summary Minutes: AWERB (PPL Review meeting)

Status: Chair approved

Meeting held: 23 February 2021 at 12.30pm via MS Teams

Present
Attendees: 9, 2 in attendance, 7 by invitation and 6 apologies

1 WELCOME

The Chair welcomed the Theatre Manager to the meeting - attending as an observer.

2 NEW PROJECT LICENCE APPLICATION:

The project licence holder was welcomed to the meeting. She explained that she was applying for a project licence to test the potential of exosomes to deliver new targeted therapies for treatment of human disease. The project would focus on the treatment of rare diseases. As it had been a few years since she had completed the Home Office module courses, she had arranged to attend the new project licence holders and E1&L courses in March.

The following comments were raised by AWERB.

- The Non Technical Summary (NTS) should be amended to make it clear which were the two main disease areas that were being looked at.
- A number of *in vivo* studies were already being run– those studies would be used to inform these studies. This information should be included in the project licence to demonstrate the background knowledge and experience of these models already held.
- There had been a swap in terminology from extracellular vesicles (EVs) to exosomes. This made the licence confusing as it was unclear if they were different or the same thing. It was clarified that the licence had initially started as exosomes, changed to EV, but was being changed back to exosomes. Exosomes were a subset of EVs of a defined size and they would be focusing on this particular population. These were key for cell to cell communication in the body. This would be explained more clearly in the project licence.
- The expected severity categories for these two models were severe for one and moderate for the other. Currently it was not clear what phenotypes these animals could experience and go through. The licence lacked a typical experience of an animal. There was wording about monitoring but this was also vague: what was being looked for; how monitored; what points led to expected adverse effects and the humane end points needed to be added. For the humane end-points only weight loss that might be experienced seemed to be mentioned. It was important to include what other humane end points might be seen. This would be added to the licence.
- Project harms section: There was a sentence that rats might be used if a difference between mice and non rodent species was observed. Why choose rats though? The project licence holder explained that this was from the first version of the project licence where she had been unclear

about how specific she needed to be. She would amend this section to explain that rats were required due to their size. For example to look at biodistribution by CNS routes of administration, the mice would be too small for the sampling, so rats would be used instead. CNS would be used for those cases around platform development where they wanted to understand the differences between routes of administration, where slightly bigger, healthy animals were needed that were not going into a disease model.

- The section on monitoring around the time of dosing and blood sampling needed to be expanded to explain how these animals would be monitored through the study.
- When going through the protocols there was mention about controls and targeting the loaded exosomes so finding the best way to deliver their cargo. However, in the studies there were no controls where there were just compounds or cargos on their own without EVs being involved. How could a comparison be done? More information should be included about how difficult it was to deliver these cargos without these EVs and exosomes so that it was possible to get an idea of why this needed to be looked at. This should go into the background of the project section.
- In general the project licence had a good approach, however there were some areas that needed more detail.
- More information about the dietary supplementation that gave the mice a better quality of life for longer was requested. Did the project licence holder know what the phenotype was with supplementation: was it still relatively normal or could there still be a number of adverse effects? The project licence holder explained that she thought the animals still had the same phenotype, but it was not quite as severe. The supplementation also mainly eliminated the risk of sudden death as welfare end points would be used before that happened. As it was a rare disease not many people were working on this, but it was known there was reasonable variability between studies, so it was a case of closely monitoring and knowing the signs to look out for and being prepared to modify the study design if things were met earlier or later.
- There was a question around the sampling and whether this would be done around the euthanasia. The project licence holder explained that this would be done if a beneficial effect of the treatment was seen, such as going onto live for another 10 weeks. Sampling would therefore be done at a later time point. AWERB asked about the potential sudden death and adverse effects: what were they measuring for during that time period: was it how long they survived for or looking for clinical signs? The way the study was designed it was to measure enzyme activity from terminal samples, but it was possible they might gain information about survival rates that could inform future studies.
- It was pointed out that blood sampling from juvenile pups was difficult at the best of times. How much blood would be taken from these young animals? This would only be done if there was a substantial effect of the mice survival.
- What were the typical numbers in relation to injections and sampling? It would be useful if this could be included in the typical animal experience section. The project licence holder advised the majority would just have a single injection. Future studies could not be designed until the duration and effect was known as this was the first time this was being done. Within the next couple of months there should be data available from other studies that could be used to inform this. For repeat dosing it was anticipated that this could vary between every 3 days to once a month. There was quite a variation as it depended on how long the effects lasted for but this would not be known until the *in vitro* and *in vivo data* from the existing studies had been obtained. AWERB suggested that a typical maximum should be included in the typical animal experience as otherwise it was difficult to assess what an animal would experience. This would just be used as an indicator.

- Euthanasia method: AWERB asked why CO_2 and decapitation was specified as the euthanasia method rather than a normal schedule 1 procedure. The project licence holder confirmed that she had based it on another study but she was happy to change this for all the protocols.
- AWERB asked about the EV production. Were they purely produced *in vitro* or by using animals in some way. The project licence holder confirmed that they were produced from human derived cell lines and were synthetically manufactured.
- For the first time that they were administered no adverse effects were expected, however would they have previously been trialled in animals? It was confirmed they would have been tested in animals previously. There would be different batches but have followed the same manufacturing controls. There might be some variability in the research scales so there could be some small differences in the batch processes but all would have had the same testing that would be expected before going into animals. This should be added to the licence as it was not clear that they been into animals before.
- It had been specified that these were naturally occurring vesicles but were species dependent. However the intention was to look for human vesicles in animals. Was there any issues in doing that, particularly if dosing them long term in terms of immune response? Had that been studied already? All the studies done to date had not shown any immunotoxicity or other major toxicities either. They had done studies in both mice and non-human primates and they had not seen anything particular significant. Had they looked for antibodies against them? These vesicles needed to bind to cells in order to release their cargo so having a blocking antibody would prevent that from happening. The project licence holder said that was one of the reasons for using exosomes, as that should not happen. The majority of the studies done were short term though and they had not had anything they would not expect but they were working with others to develop the assays to look particularly for that so it could be thoroughly characterised. They were aiming to get into clinics on quite fast time scales so would need the data for that. It needed to be known before any longer term studies in animals were embarked upon.
- How would all the objectives and project plan work together? AWERB were particularly interested in the stop/go points. Presumably there was something that needed to be looked out for at a particular level or an adverse effect before progressing into the disease model? The project licence holder explained that there was a diagram that she had wanted to include that made the stop/go decisions clearer. She would circulate it separately. In summary, they were looking to confirm that they were targeting the right organs with enough therapeutic cargo to see an effect to then go into a more GLP toxicology type study.
- How would in silico modelling be approached further along in the project? The project licence holder advised that currently they had minimal data as these were the first projects but there should be parallels such as cell type producing the exosomes or using the protein cargo, so things that would become consistent across the different projects. She therefore hoped that they could use that information to do that sort of modelling piece. They were also hoping to look at *in vitro* models and then use those. None however were quite validated enough to be used for the initial studies. She was hopeful that within the next 12 months they could start to test more things in those models and then follow how those data looked to see if it could inform future projects. She would add this to the 3Rs section.
- Experimental design: data were being used from the studies that had been outsourced. Could these data be used to calculate sample sizes to avoid having to do pilot studies? Ideally, pilot studies should be used to look at the logistics and flag up things that had not been prepared for before starting the main study, so that it could inform experimental design. The pipeline project ones that were due to finish April/May time would inform things – the variability was currently not known so it was currently hard to design group sizes. The project licence holder was aiming

to use the pilot studies for some of the routes of administration that had not been tested and so did not know what the data would look like yet. It was suggested that this be added into the experiment design sections to explain that there were some ongoing studies on another PPL which would be used to inform on variability for design of the pilot experiments.

- What was the basis for the hierarchy of administration? Was it a scientific basis or welfare basis and going with the least harmful way first? For example intra-muscular was mentioned as a route of administration but this AWERB preferred to avoid that if possible as it could be very painful for the animals. The project licence holder explained it would depend on the project. For some it would be IV as that was used clinically; for others it would be IP; some could be influenced by the size of the animal and the actual ability to be able to dose that animal.
- AWERB asked about the bioavailablity of the different routes and comparing them. If the aim was to end up in the clinic was it comparable to start in an IP and then go into an IV – was it translational? Ideally they would use IV, but if it was too challenging to use in the animal model then they would need to think about what the most appropriate route was. Would bioavailablity studies work to see if those routes were comparable, as something might be done IP which was completely different to IV. The project licence holder said that was why they were also doing comparisons with healthy animals so would know if biodistribution was comparable – if it wasn't they would not choose that route. The project licence holder would include justifications of why these were the most appropriate model to use at the end of each protocol.
- What controls were in place if the mice experienced seizures in the cage? What data had been gathered so far? The project licence holder explained that the seizures shouldn't occur as the humane end point would already have happened by then.
- What skill sets or support was the project licence holder's team providing? The project licence holder confirmed they would be overseeing the studies, however the majority of the support would be provided by the BSU staff.

The project licence holder was thanked for attending the meeting. Overall AWERB thought the proposed work was interesting but that more information and background needed to be added. A summary of the points raised at the meeting would be provided so that these could be addressed. Once the project licence had been amended it would be circulated for a further review.

After the project licence holder had left the following points were made:

- The project licence had come to AWERB at too early a stage as it was lacking in detail about what the animals would go through to enable a thorough harm benefit analysis to be done.
- It was noted that under the measures that would be used to optimise the number of animals that were planned to be used, mention was made about using a natural history study. More detail was needed about what exactly was planned.

3 PROJECT LICENCE AMENDMENT

A request to amend a project licence had been received for the following changes:

1. Skin closure: in some animals, they had observed chewing of sutures after surgery. In discussion with the NVS and HOI, they had trialled closing the skin by using a temporary suture and tissue glue, which resulted in good wound closure with no evidence of wound chewing post-surgery. They therefore wanted to amend the project licence to include this as a refinement.
2. Addition of therapeutic agents: they wanted to add some therapeutic agents following discussions about a potential collaboration with an industrial partner who was interested in looking at the effects of nitric oxide on tendon healing. Trials had shown that this could

improve tendon healing but that this could result in side effects. The company had therefore developed a new way of delivering the product that could be used in the localised area that they wanted to test.

3. Update the number of blood samples taken: this was linked to the 2nd amendment: the initial study would look at pharmacokinetics which would require repeated blood sampling.
4. Addition of gait analysis: this would look at the healing as a non invasive method to see how much the rats were favouring the limb that the injury had been induced too.

A query was raised about the blood sampling which mentioned anaesthesia. Using anaesthesia could end up being more stressful to the rats than the blood sampling. The project licence holder explained that one of the formulations would require regular dosing with the drug and blood sampling at the same time (on 8 occasions) so it would be better to anaesthetise in order to do all this at the same time. It was confirmed that this would only be used when needed. It would not be done for all blood sampling. The same applied for the intraperitoneal and subcutaneous injections. Cadavers had been used to trial the technique, but the animals might have to be anaesthetised to ensure that the injections were going into the right place.

How often would the gait analysis be done? This would be done 2 to 3 times the first week after surgery and then on a weekly basis. The first gait analysis would not be until the following day after the surgery but would be within 24 hours. This would be added to the project licence as it had an impact on the animal welfare.

AWERB asked for further detail around the administration of the nitric oxide and what the formulation was of the product. Nitric Oxide was very short lived in the tissues and that it was the pro drug that released the nitric oxide. Was it this that was retained locally? The project licence holder explained that she did not have the full details from the company but they had two different formulations. One was fast acting and one was slow acting. The idea was to get coagulation around the tendon to hold the drug there as well as doing some blood work to look at how that stayed local and how much did get systematically in the blood as well – hence why the blood sampling was needed. AWERB advised that one of the problems with nitric oxide was tolerance – they did need to know about the pharmacology and whether it was dependent on enzymatic breakdown to produce the nitric oxide in which case would expect tolerance to happen locally or whether it was spontaneously decomposing into nitric oxide locally and retaining the drug and it would be a chemical reaction that was gradually happening. This was important to understand as tolerance was a real issue. The project licence holder would double check with the company as it would influence the experimental design to know what the pharmacology was doing in terms of the dosing.

A query was asked when the data would be obtained from the company. It was expected relatively soon. As it could influence the experimental design, if tolerance was likely to develop then only the acute effects of the product could be looked at. However, it should be possible to write the project licence sufficiently broadly to allow more than one type of nitric oxide donor to be studied, if that was what wanted to study. The results would be needed through before the study could be designed to have a look at this.

It was pointed out that early on in the amendment there had been mention made of different locally acting agents but that in the protocol it just specified nitric oxide. Were they looking to be more broad in the licence? The project licence holder said she was unsure how specific or broad to make them. They were interested in nitric oxide at the moment but would it be more beneficial to make it wider in case they needed to study other elements later on so a further amendment would not need to be submitted further down the line? She was advised that generally being more specific was better for AWERB in terms of reviewing the licence, but of course if things changed, then amendments needed to be submitted. So it all depended on how likely that was and how regular that a new agent would need to be trialled.

It was confirmed that the following areas needed to be expanded on:

- Gait analysis frequency
- Experimental design: consider what want to get out of this.
- A query was raised as to whether the project licence should be amended to remove the option of using an absorbable suture or should it be kept in along with the change to the new refined method. It was recommended that both be kept as if the tissue glue was not effective, it meant that there was still an alternative that could be used.

The project licence holder was thanked for attending.

AWERB confirmed that they were happy with the proposed amendments which seemed to be straight forward and well thought through and included refinement aspects

4 PROJECT LICENCE AMENDMENT

A request had been received to amend a project licence. Following feedback from the regulatory agents, the project licence holder would like to introduce an amendment to the project licence to do some behavioural studies in their mutant lines. These would be added as steps to protocols 2 and 3.

The following queries/points were raised:

- Some of the animals were prone to seizures and other side effects. Did they foresee any problems or more adverse effects from doing behavioural tests in different environments, that wouldn't be see in a more healthy animal? This would need to be monitored and the phenotype refined if need be. They were not expecting to see anything though.
- What was meant by quantify? Were they looking for 10% of mice to show this behaviour? And if yes, was that 1 in 10 mice or 10 out of 100. How many did they need to go through this procedure? The first protocol would be run with 10 mice and these would be used as an end point in the proof of principle studies. They were looking for a clear phenotype that they were able to quantify.
- More detail was needed on the study design. For example was it just comparison between mutants and mutant + treatment? Justification for the potential number of mice that were needed to go through these procedures were also required. It was explained that for the first protocol the focus was on proof of principle to make sure there was a phenotype, which would then be used as end points for the study.
- A query was raised whether the approach to behavioural testing should be included in the project licence. It depended on what was included in the experimental design and how specific that was.
- How were the experiments chosen? Had they used them before? The objective was to test learning and short and long term memory so it needed to be possible to measure responses and use that as the study.
- The project licence holder asked could they assume that if there was no reaction then the animals were not experiencing any stress? It was pointed out that this assumption could not be made. For example animals found being handled very stressful but they did not always react.
- Some strains of animals did learn more quickly than other strains. Were there any expected seizures and adverse events that might crop up? The project licence holder advised that nothing had been seen in the strain they were planning to use but that if anything appeared they would take this into account.
- The proposal was to use Wild Type animals in the pilot. This should include a proper analysis of what the animals would go through.

The project licence holder was thanked for attending the meeting. There were several areas that needed to be re-worded and clarification sought particularly:

- duration and how to approach it
- the cumulative effects

- a more clear description of what the animals would experience and a stop/go plan.
- On a practical front they needed a clear plan on how to do this and set up as a pilot study on the cognitive behaviour test. This needed to be planned correctly and the stop/start decisions clearly set out so that AWERB was reassured that if certain points were encountered the study wouldn't continue.

The project licence would be reworded and resubmitted for review.

5 MINUTES

The minutes of the standard agenda items meeting held on 11 February 2021 were approved.

6 ACTION LOG

6.1 Item 3: Proforma PPL comments form (11 February 2021)
Meeting scheduled for 16th March to discuss this comments form.

6.2 Item 8: Assessors list review (11 February 2021)
The assessors on the list were being contacted to check that they were still happy to be listed.

6.3 Item 2: update on Camden ponies (26 January 2021)
An update on the pony that was to be retired was given. It had been decided to see how she found being involved in teaching at the Hawkshead campus, so she had been included in sessions the previous week and had been fine. Equine were therefore keen to give her a trial for a couple of months to make sure that she continued to fit in and was happy. She would therefore be officially retired from Camden and a replacement pony sourced.

7 REVIEW OF MEETING FORMAT

The Chair asked AWERB how they had found the meeting. Had it worked better in splitting the meetings into two and just focusing on the project licences at this meeting? The consensus was that it had as it gave an opportunity to really get into the details of the licences and to ask more questions.

8 DATES OF MARCH MEETINGS

These were arranged for:

- 12 March: 1pm – AWERB standard agenda items meeting
- 23 March: 2pm – AWERB PPL reviews meeting

Secretary
09 March 2021