ANIMAL WELFARE
AND ETHICAL REVIEW BODY

Minutes of the meeting held on 14 March 2019

Present: [redacted]

In attendance: [redacted]

Apologies: [redacted]

1. Minutes

Agreed: That the minutes of the meeting held on 17 January 2019 were approved.

2. Matters arising not covered elsewhere in the agenda

2.1. Membership

a) [redacted] was welcomed to his first meeting
b) [redacted] has resigned from the committee

2.2. Requirement for confidentiality agreement for AWERB members external to the University.

a) It was reported that there is no requirement for a confidentiality agreement.

b) The AWERB members present at the meeting discussed the confidentiality agreement and agreed that they did not feel that a confidentiality agreement was appropriate in this case.

3. Update on applications considered at the 17 January 2019 meeting

3.1. The revisions for the 3 project licence applications and 1 amendment considered and provisionally approved by AWERB at the previous meeting had all submitted revisions which had been approved, and subsequently received Establishment Licence Holder approval. The applications had been either approved by the Home Office or submitted for approval.

4. NW region AWERB hub

4.1. The notes from the NW AWERB hub will be circulated to AWERB members once they have been finalised.

4.2. [redacted], as a lay member, said that the meeting had been very useful and would like to encourage the future host institutions to invite lay members.

4.3. [redacted] thought that having a lay group forum on the hub website would be beneficial.

5. NC3Rs Regional Programme Manager update

5.1. The post has been filled but a starting date has yet to be determined.

6. Report on amendments processed from 21/12/2018 to 22/02/2019

The following amendments were approved by the executive committee.

6.1. Amendments to Project Licences

[redacted], Regulation of Glomerular Barrier Function in Health & Disease.

[redacted], Generation, Breeding & Maintenance of Genetically Altered Rodents.

[redacted], Extracellular Matrix in Development & Disease.

[redacted], Understanding Inflammasome Dependent Inflammation.

[redacted], The Role of Inflammation in Cerebrovascular Disease.

6.2. Amendments to Project Licence [redacted]; Generation, Breeding and Maintenance of Genetically Altered Rodents

[redacted], Generation of Ngn2-TagBFP Mouse Line.

6.3. Amendments to Project Licence [redacted], Generation & Breeding of Genetically Altered Rodents

[redacted], To Create a Foxc1$^{tm1(EGFP)TS}$ Mouse Line Using CRISPR.

6.4. Applications for secondary availability for new or current project licences

Considered: A completed AWERB2 form, PPL application, letter of compliance for working within the BSF,

Interviewed: [redacted]

Discussed:
- Most of the work on the licence will be carried out at the University of Sheffield.
- The secondary availability is for work to be done at The University of Manchester where some of the strains of mice are located. Specialist staff from the University of Sheffield will come to the University of Manchester to carry out the work.
- Single housing will not be done without good reason and if it occurs it will be declared in any publications. Approximately 5% of mice would be singly housed over the course of the Project Licence.
- In Protocol 1 (1000 mice) some of the potential adverse effects of arterial ligation appear to approach substantial severity. Of the 1000 mice, [redacted] would expect from her experience that 2-3 mice will actually experience this level of suffering.

Revisions:
- Please can you explain the likelihood of the possible compound adverse effect of multiple treatments that animals could receive and how often this will occur.
- The NTS would benefit from some minor changes. Lay members of the Manchester AWERB ([redacted]) would be happy to support you in making the changes.
    - It is definitely written in lay terms but the term "sacrifice" is still used and there are some spelling errors and sentences that need a bit of clarification.
    - The first sentence states that you do not expect any adverse effects but then there are some listed. Perhaps this sentence could be removed.
- Please confirm that all investigators, particularly the non-University of Manchester investigators, are familiar with, and will comply with, the University of Manchester Policy on the use of Animals in Research including in particular the ethical obligation to make all the findings (including negative findings) from your research publicly available.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

7. Applications for New Project Licences

7.1. [redacted] Novel Targets for Anti-Epileptic Drug Design ([redacted])

Considered: A completed AWERB2 form, PPL application, and minutes from the Local Management Committee Meeting

Interviewed: [redacted]

Discussed:
- AWERB would like a discussion to take place between the statistician and applicants to determine the most appropriate experimental

model, including the number of animals in the control and drug groups and the level of seizure induced on the Racine scale.

- AWERB discussed the number of control animals to be used and if there was a need to use one-to-one matching for the control animals and those given the compounds. The applicants explained that slight differences between batches of drugs and animals can occur which could affect the results.

- The Committee asked if there was the possibility of including an in vitro stage between the work already carried out in flies and that proposed in the Project Licence application, for example the use of stimulating seizures in brain slices from mice. The applications explained that the expertise needed for these experiments are not present in his lab, and the focus of the experiments in the Project Licence was about the in vivo properties of the compounds.
- The Committee discussed with the applicants if the PTZ-induced seizure model was required (Protocol 2) given that the applicants describe a direct correlation between Pumilio expression and seizure recovery. The applicants explained that in their opinion to show the compounds are protective the PTZ-induced seizure model was required.

Revisions:

- The Committee had concerns with the requirement to put mice into full tonic-clonic seizures (Racine point 5) to show compounds can prevent that level of seizure severity. While taking into account the ability to produce robust, meaningful and publishable results, the committee would like the applicants to clarify if the study could be carried out to a maximum of Racine point 3/4. Members of AWERB discussed if using more animals at a lower severity would be more ethical than fewer animals going up to Racine 5. Please contact [redacted].
- The NTS requires some minor changes, in collaboration with the lay members of AWERB if the applicants would find this useful [redacted])
    - The Aims and Objectives could benefit by a more succinct and simpler description after the sentence "Thus there is a clear clinical need for better AEDs."
    - There are still some technical terms used which could be altered e.g. 'Racine stage', 'proconvulsants' even perhaps 'power calculations' which will not mean much to the non-specialist.
    - Is there a more lay expression for 'identification of novel biology' - is this the same as identifying novel targets? Is this about better understanding the underlying biological process of seizure?
    - Is there a clearer way to express what 'neuronal and neuron homeostasis activity' is so the reader can understand what is (potentially) being manipulated?
    - In the expected adverse effects and severity section it implies 'stage 5 full-blown tonic seizure' is severe but does not give any indication of the level of suffering expected during other forms of induced seizure; please can some description be used.

- The description of behavioural change is very clear.
- Reduction section
  - The P values are potentially not necessary.
  - The second sentence could be omitted or reworded to "Test compounds manifesting potential toxicity on screening will be deselected. Pilot studies of 5 mice would ensure that compounds with potential activity against the known targets would be used." The applicants should discuss with the BSF staff if this should be moved to the Refinement section
- Refinement section
  - This could be made simpler and shorter.
  - The first and second sentence could be changed to "The mouse is the accepted model for research in seizures and identifying new AEDs."

- The applicant states "We will perform all work in accordance with the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines)". While the spirit is commendable, please note that ARRIVE provides guidelines on reporting and not primarily on the conduct of the studies themselves. Perhaps the applicant intended to say something along the following lines: "In accordance with the University of Manchester's policy on animal use, both positive and negative findings will be published. All studies intended for publication will be designed so that the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) can be properly addressed in ensuing publications."
- Please can you clarify in your response letter if there are plans to test the compounds in female mice. Additionally please can you state if there are gender differences in epilepsy between the sexes.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

7.2. [redacted], Zebrafish Models for Investigating Cancer Formation/Progression, Immune Responses & Immunotherapy ([redacted])

Considered: A completed AWERB form, PPL application, and minutes from Local Management Committee Meeting

Interviewed: [redacted]

Discussed:
- AWERB thought the presentation was excellent and documentation was very well prepared.
- AWERB would like the applicant to develop a publication plan to disseminate any refinements arising from the use of fish rather than mammals in the study of cancer. The applicant's lab has participated in open days with the public, and his work has been in the press and on the radio, and would like to do more. AWERB are fully supportive of this refinement and would be happy to discuss ways in which [redacted] can promote his work. [redacted] is more than happy to meet with [redacted] regarding placing the work on the externally facing website University of Manchester website.

Revisions:

- The NTS would benefit from some minor changes which are listed below, but AWERB thought that it was generally an excellent lay summary:
    - Please expand briefly on what CART is and expand on the positive use of fish rather than mammals for the non-specialist.
    - AWERB thought it may be appropriate to mention under refinement the plan to use 'pilot studies' to guard against unanticipated toxicity. Please discuss with the BSF staff if this would be a refinement.
    - Please can you briefly describe what melanoma is and why it is important to understand.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

7.3. [redacted], Mechanisms of Breast Development & Tumourigenesis ([redacted])

Considered: A completed AWERB2 form, PPL application, and minutes from the Local Management Committee Meeting

Interviewed: [redacted]

Discussed:

- The tumours in this Project Licence should be non-metastasising, which is a refinement.
- Analgesia will be used after surgery, which is a refinement.
- The applicant was asked how many mice are expected to develop lung tumours. [redacted] explained that zero mice are expected to develop lung tumours however the use of knockout mice mean that approximately 20 mice over the project licence may develop a metastases. Any animals that exhibit signs of metastasis will be humanely killed.

Revisions:

- The NTS would benefit from some changes, and the lay members of the committee ([redacted]) are more than happy to meet with the applicant should it be felt that this would be useful:
    - Aims and Objectives: the second paragraph would benefit by being simpler and succinct. A suggestion would be: 'The aim of this project is to confirm the in-vitro findings that the physiological signalling process which regulates cell motility and growth in normal tissues closely reflects the true biology of cancer and its inhibition changes Br-CSC properties ultimately preventing them from forming tumours. We plan to assess how genetic deletion of different factors that are involved in the signalling process affects tumour growth in an animal model that is genetically prone to developing breast cancer. Because these...'
    - Potential benefits: Third sentence: We would suggest a change to 'Demonstrating that a permanent deletion of certain components of the signalling process inhibits...'
    - Please carefully check the application for typographical errors, for example in the NTS refinement section instead of

'We plan to use mouse because' should read 'we plan to use mice'.
    - The use of acronyms should be kept to a minimum, and all terms should be fully explained the first time they are used.
    - In the reduction section, the final sentence requires some revision to make it suitable for a lay audience.
    - Please briefly explain 'metastases' for a lay audience.
    - Please replace or remove the use of the word 'horror' on page 41 of the project licence application.
    - Please can you clarify if it is 'known' or 'likely' that BrCSCs are the cause of tumour recurrence.
    - We suggest that the sentence 'Our initial analysis showed that a genetic deletion of Rac1 in the breast or other components of the Rac1 signalling does not compromise the animals' health' is removed from page 42 of the project licence application.
    - The replacement section requires some revision to ensure that it does not contain non-relevant information for that section such as objectives.
- The possible number of injections to be given, dosage and how often is often not consistent. Please ensure this is mentioned for all injections.
- Please be specific about weight loss; it could be interpreted as **in** some mice **it is** going to **be** greater than 20% weight loss.
- There are inconsistencies in the forms for the number of animals to be used. Please ensure they add up correctly.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

8. Any other business

8.1. Change of order of the agenda and maximum number of PPLs per meeting

a) In future meetings the Project Licence Applications will be considered before any other licences
b) Only 3 Project Licence Applications will be considered at each meeting.

8.2. Home Office Inspector and Establishment Licence Holder meeting

a) [redacted] met with the HOI and PEL and the feedback from the HO was very good.
b) The number of visits will be reduced.
c) [redacted] reported that the last three Project Licence applications that the HO have considered from the University of Manchester have required no changes.

8.3. Animal Research Nexus

a) [redacted] discussed the Animal Research Nexus (AnNex) and explained the aims of the AnNex are to deliver new research and engagement to enhance the understanding of the connections between science, health and animal welfare.

8.4. AWERB away day/afternoon

a) An away afternoon for AWERB members would be organised and potential dates for this would be circulated by the Secretary for AWERB.
b) Lunch would be provided from 12-1pm with the meeting taking place between 1-5pm.
c) AWERB members were asked to send any agenda items to the Secretary for AWERB. Depending on the number of items it may not be possible to discuss all of them at the meeting, but future away days would be scheduled.

The next meeting will be on 16 May 2019 at 10am-12pm, in [redacted].

Dates of meetings for the 2018/2019 academic year are:

[redacted]