**CONFIDENTIAL MATERIAL**

THE UNIVERSITY OF BIRMINGHAM

BIOMEDICAL ETHICAL REVIEW SUB-COMMITTEE (BERSC)

3rd September 2015

# MINUTES

15/09-02 Minutes

The minutes of the meeting held on 13th August 2015 were considered by the Committee and were approved subject to amendments.

15/09-03 Matters Arising

*Minute 15/06-08 – Educational remit of BERSC*

Both the educational remit of BERSC and the matter of interim reviews will be carried over for discussion at a future date.

15/09-04 Chairperson's Items

There were no Chairperson's items to report.

15/09-05 Verbal Reports from the Director of BMSU and Named Persons

The Named Persons had no issues to report.

15/09-06 Report from the Fast Track Procedure

The fast track procedure is up-to-date, and there are no outstanding issues.

15/09-07 Project Licence Proposals

15/09-07-1 Application Ref TBA – Does steroid production in inflammatory arthritis cause joint destruction, muscle wasting and bone loss?

The objective of this project is to discover whether the joint destruction, muscle wasting and bone loss observed in rheumatoid arthritis are due to the effect of steroid production on the cell communication network known as 'Wnt signalling'.

The PI gave a presentation explaining the application to the Committee.

It was queried whether the PI had read a recent article by Hawkins et al (2015)[1] on refinements in rheumatoid arthritis research which considers ways of minimising disability and pain. The PI confirmed that he was aware of the article and had read the abstract; the issues considered in this article have been taken into account in the scoring systems to be used for this work (e.g. the incorporation of assessments of pain expression, mobility and social isolation).

It was felt that the current description of the scoring system within the application is confusing. Also in relation to scoring, it would be preferable to minimise the number of times the animals need to be handled and to instead carry out as much scoring as possible via observation.

Further explanation is required of the proposed humane endpoints for this study. It was explained that the Home Office Inspector had already raised this issue, and additional information had been included in a more recent version of the application.

In the application, it is stated that pain relief will be given 'under the advice of the vet'. The Committee felt that it would be helpful to provide further details about the proposed pain relief regime. As opiate analgesia may have an anti-inflammatory effect, controls will need to be in place for this. Some concerns were raised in response to the suggestion of self-administered analgesia; particularly, the danger that if an animal is in pain, it may not be sufficiently mobile to self-administer. The PI was advised to consider the use of long-acting analgesia, as discussed in the article by Hawkins et al (2015).

It was clarified that whilst observation of the animals for scoring purposes will take place three times a week, more general observation of the animals and administration of analgesia will take place daily.

The PI was asked why animals will need to be monitored in the PhenoMaster cages for 48 hours, singly housed. The PI explained that

[1] Hawkins, Penny and Armstrong, Rachel and Boden, Tania and Garside, Paul and Knight, Katherine and Lilley, Elliot and Seed, Michael and Wilkinson, Michael and Williams, Richard O. (2015) 'Applying refinement to the use of mice and rats in rheumatoid arthritis research', Inflammopharmacology, 23(4), pp. 131-150. (10.1007/s10787-015-0241-4).

this time period is informed by the previous work of others and 48 hours has been treated as the standard, but the PI will explore whether this time period can be reduced. An enriched environment is provided within the PhenoMaster cages. It was noted that the animals will only be placed in PhenoMaster cages as a one-off event and it will be the last thing which happens before they are humanely killed.

The Committee queried why work with human tissue will be carried out in parallel with the proposed animal work, and it was explained that the work with human tissue will inform objective three of the animal work.

The non-technical summary should be clarified in relation to human studies. It is not clearly explained why there is a need to use animals. It should be stated that it would not be possible to carry out trials in humans without having previously obtained data from a well-established animal model.

With regard to the non-technical summary, it was suggested that the current proposal to administer Remicade may give rise to confusion because the experiments require inflammation to be induced, while Remicade is an effective treatment for rheumatoid arthritis and can be expected to reduce inflammation. The PI clarified that Remicade will only be used to help maintain the required breeding programme and will not be used as part of the proposed experiments. It was also queried whether the existence of an remedy such as Remicade negates the need for further research in this area; it was explained that Remicade is primarily an end-stage drug, and is not suitable for use in all patients, meaning that further research is still required.

The number of animals stated in the non-technical summary should be revised to show the split between breeding and experimental purposes. Also, an indication should be given of how the stated numbers were determined. It was suggested that the number of animals to be used for breeding is currently too low and should be increased.

Where possible, scientific or technical terms in the non-technical summary should be revised or explained to make the text clear for a lay reader.

In the non-technical summary, it was noted that the statement that mouse models, 'allow us to perform in vivo studies that would be otherwise physically or ethically impossible in human cohorts' has been rewritten in a more recent draft of the licence to state that the proposed in vivo studies 'are not possible' in a human cohort.

The Committee queried whether both a global and a targeted knockout model are required. The PI explained that both models are needed because a key aspect of the work is subtractive phenotyping. It was noted that neither the global nor the targeted knockout models display

an adverse phenotype without inflammation being present. It was acknowledged that the targeted knockout is the better model, but the global knockout is important too as it informs the targeted work and allows work to take place over a shorter time period.

All of the work included in this licence application is funded by an ARUK Career Fellowship.

The PI was asked to justify the use of a polyarthritis model, when the previously mentioned paper by Hawkins et al (2015) advocates the less severe, antigen induced model. It was explained that the antigen induced model does not create the same systemic involvement as occurs in the polyarthritis model. The reproducibility of the model is important, and the collagen antigen induced model may not be the first choice with respect to this.

After the PI left the meeting, the Committee continued its discussions.

It was recommended that further information should be included in the section about the PI's relevant knowledge, skills and experience.

*Resolved that:*

The revisions discussed above will be made and the revised application will be circulated to the Committee via SharePoint. Once the Committee is happy with the changes, a recommendation will be made by the Committee Chair that the Establishment Licence Holder submits the application to the Home Office.

15/09-07-2 Application Ref TBA – Regulation of leukocyte recruitment during acute and chronic inflammation

The aim of this project is to understand how immune cell derived agents (e.g. PEPITEM) and tissue-resident cells (e.g. fibroblasts or mesenchymal stem cells) control the movement of immune cells in health and disease.

The PI gave a presentation explaining the application to the Committee.

It was recommended that the researcher reconsiders the wording 'allow us to perform in vivo studies that would be otherwise physically or ethically impossible in human cohorts' in the non-technical summary, and further justification is needed for working with animals rather than humans. In relation to the work with human cells in vitro, it was explained that very few cells are used and that this cannot reflect the systemic effects seen in a whole animal.

In the section 'Replacement', it was suggested that the PI should amend the phrase 'There are no other in vitro or in vivo alternatives to

this work', to 'There are no other known in vitro or in vivo alternatives to this work'.

In the section 'Refinement', additional and more specific information should be given about the refinements to the models carried out at the University of Birmingham.

In the section on adverse events, it should be clarified that ongoing analgesia will be provided.

The current version of the scoresheet to be used was provided to the Committee for information.

In the chronic model, the inflammation will be of variable onset which makes it more difficult to determine an appropriate regime for analgesia. The PI explained that with the help of the NACWOs, the animals will be observed daily to ensure that analgesia is given as required and analgesia will be first given at the point where arthritis is induced.

It was queried whether the use of Freund's adjuvant is really necessary. If it is necessary, the justification for it should be strengthened in the licence application. The Committee was informed that very few of the reported adverse effects of Freund's adjuvant have been seen within BMSU, and it was felt that this may be a matter of administration technique. The application should be clarified to state that Freund's adjuvant will only be used once on any animal.

Further justification was requested for the prophylactic administration of agents, when this would not be done in a human patient. It was explained that experiments involving prophylactic administration will only be carried out to establish whether the agent can prevent disease or reduce symptom onset; this is unlikely to progress to therapeutic experiments, although a small pilot study may be carried out.

Imaging will not be used in the arthritis models, but will be used for other parts of the study. It was explained that some institutions are using multiphoton imaging for similar work, but BMSU does not currently have the necessary equipment for this.

The PI was asked to ensure that the number and frequency of injections is clearly stated in the application.

The application should be amended to ensure that it consistently states whether wild type or genetically altered mice will be used.

There was some debate about whether ulceration should be a stated endpoint on the licence, and a distinction was made between ulceration related to a tumour and that related to the use of Freund's adjuvant.

After the PI left the meeting, the Committee continued its discussions.

Regarding the section about the PI's relevant knowledge, skills and experience, although it is understood that the PI has little directly relevant experience, this should be explained rather than leaving the section blank.

A secondary contact, in case the PI is for some reason unavailable, should be stated in the application.

*Resolved that:*

The revisions discussed above will be made. Once the Chair is happy with the changes, the Chair will recommend that the Establishment Licence Holder submits the application to the Home Office.

15/09-07-3 Application Ref TBA – Repairing the injured retina after blunt ocular trauma

The objective of this project is to determine changes that occur after blunt trauma to the eye, which is connected via the optic nerve to the brain and relays information about vision. The researchers are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, and the lack of axonal regrowth that follows traumatic eye injuries.

The PI gave a presentation explaining the application to the Committee.

It should be emphasised in the licence application that anaesthesia will be given to the animals prior to injury to the eye.

The Committee queried whether the force required to cause the injury to the eye will lead to swelling. The PI explained that there may be a small amount of localised swelling, but no bleeding, breaking of the skin or rupturing of the eye. Any swelling is expected to go down quickly.

Whilst it is possible to scale down the proposed work from rats to mice, and although mice have been included on the application in case there is a need for work with genetically altered animals, mice are unlikely to be used.

It was queried whether it is necessary to allow animals to recover from the anaesthesia after the injury, or whether they could be humanely killed without allowing them to wake up. The PI explained that the injury will be carried out under anaesthesia, but the animal must be allowed to waken in order to be able to monitor the effects over several days.

The 3Rs section should be strengthened and refinements made during previous related work by another researcher at Birmingham should be mentioned. The refinements should include matters relating to pain relief and monitoring.

In the section on adverse effects, where necessary the wording 'potential harm' should be changed to 'actual harm'.

Within the application, references to 'globe' should be changed to 'eyeball'.

It was queried whether it is possible to use a blast of pressurised air rather than a projectile to create the required injury. The PI explained that this has been considered, but is not logistically possible. Also, the damage caused to the eye would be the same using either projectile or air blast.

It was felt that the term 'lesion' is not a good descriptor of the injury, and should be revisited; 'bruising' was suggested as more appropriate.

The PI confirmed that in previous similar work, it has never been necessary to humanely kill an animal following the injury and no rupturing of the eye has ever occurred.

Whilst the application allows up to eight occasions of anaesthesia, most animals involved in this work will experience around three.

If any animal develops uveitis it should be euthanized rather than attempting to treat the condition with steroids.

The PI has considerable previous experience in using electrodes to record retinal function. Such recording will only take place over one day for each animal.

Intracranial injections may be necessary if the researchers wish to use biomarkers and they will be carried out under anaesthesia. It will be clarified in the application that less than 5% of the animals will undergo this procedure.

In relation to the proposed use of scleral indentation during fundoscopy, the PI explained that indentation can give a clearer picture of the eye, but it may not be needed and is still under discussion. Also related to fundoscopy, the retina may be imaged and this will be carried out under anaesthesia.

Further information was requested about the nature of the behavioural assessments. The PI explained that these will be looking at visually guided behaviours.

The Committee asked the researcher how the mice will be picked up when the need to be placed into the maze, as lifting by the tail may induce a fear response and cause the mice to 'freeze' rather than move naturally. The PI clarified that the mice will be cupped in the hand of the researcher rather than lifted by the tail.

Further explanation of the basis for the stated sample sizes should be included in the application.

The application includes a number of possible routes for the administration of therapeutic agents. It was explained that the most commonly used route will be injection, but new routes of administration such as eye drops are under development.

It was suggested that hydrophobic sand could be used to obtain urine samples, rather than metabolic cages.

*Resolved that:*

The revisions discussed above will be made, and a revised version of the non-technical summary will be recirculated to the Committee via SharePoint. Once the Chair is happy with the changes, the Chair will recommend that the Establishment Licence Holder submits the application to the Home Office.

15/09-08 Any Other Business

*3Rs day*

Discussion of proposals for a '3Rs day' will be carried over to the next meeting.

15/09-09 Date of Next Meeting

The date of the next meeting is 1st October 2015.

## GLOSSARY

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| 3Rs | Reduction, Refinement and Replacement |
| ARUK | Arthritis Research UK |
| BERSC | Biomedical Ethical Review Sub-Committee |
| BMSU | Biomedical Services Unit |
| NACWO | Named Animal Care and Welfare Officer |
| PEPITEM | PEPtide Inhibitor of Trans-Endothelial Migration |
| PI | Principal Investigator |
| TBA | To Be Announced |
| Wnt | Wnts are secreted factors important in regulating the survival, proliferation and differentiation of mesenchymal cell populations. |