CONFIDENTIAL MATERIAL

THE UNIVERSITY OF BIRMINGHAM

BIOMEDICAL ETHICAL REVIEW SUB-COMMITTEE (BERSC)

13th August 2015

MINUTES

15/08-02 Minutes

The minutes of the meeting held on 4th June 2015 were considered by the Committee and were approved subject to minor amendments.

15/08-03 Matters Arising

Minute 15/06-08 – Educational remit of BERSC

This will be carried over for discussion at a future date.

15/08-04 Chairperson's Items

Interim project reviews

The Committee was reminded of the need for interim project reviews.

ACTION: The Director of BMSU will prepare a format and schedule for interim reviews.

Additional lay member for BERSC

It was suggested that it would be helpful to have an additional lay member in addition to those currently serving on the Committee. A particular individual with a background in ethics was suggested. It was agreed that the person will be approached and if they are interested, they will be invited to attend a Committee meeting as an observer in the first instance.

15/08-05 Verbal Reports from the Director of BMSU and Named Persons

Report from Director of BMSU:

Key issues reported were:

- ASPeL, the Home Office electronic licensing system, is currently being upgraded to allow online electronic Project licence applications (currently, only Personal licences are processed online). BMSU is an early adopter of this new system, and it is anticipated that the first online Project licences will be attempted in early September 2015. It is hoped that the new system will be helpful for applicants as they will be able to track the progression of their applications. The content of the form is not expected to change significantly from that of the current paper version, and the stated Home Office processing timescales will remain the same. Home Office feedback on applications will still be sent to both BMSU and to the applicant.

- Two FOI requests have recently been received. The data requested in one of these was provided by signposting the author of the request to BMSU's external website. The other request resulted in a televised discussion programme on the number of animals used in research. The University was asked to put forward a participant for this programme; however, instead a representative of Understanding Animal Research attended on behalf of the University sector. Feedback has since been received from the Home Office that this was a missed opportunity for the University to explain the valuable animal research it carries out to a wider audience. As an outcome, discussions are underway to bring together a group of trained people within the University who are willing to speak at such events. A number of short case studies and comments on animal welfare from BMSU animal technicians will also be added to the externally facing BMSU website.

- Unfortunately, there are still difficulties with BMSU's colony of captive-bred xenopus frogs. The animals BMSU already held that had pseudocapillaria were replaced via a new source of captive bred frogs. However, these new animals have subsequently been found to have a low level of mycobacteria. They have been put into quarantine, but as it would preferable not to hold up the work they have not yet been culled; however,

they will not be held long-term because of the small risk of zoonosis. Efforts are being made to find another breeder but this may not be feasible. Alternatively, it may be possible to purchase frog ovaries containing eggs rather than the whole animal, but this raises the additional ethical issue of killing the animals for their eggs rather than taking the eggs deposited by live animals. The Committee will be kept updated.

No additional items were reported by the NACWOs or the NVS.

15/08-06 Report from the Fast Track Procedure

The fast track procedure is up-to-date, and there are no outstanding issues. It was noted that there are some interesting applications currently going through fast track and these will be made accessible to the rest of the Committee for information.

15/08-07 Project Licence Proposals

15/08-07-1 Application Ref TBA – Repairing the damaged peripheral nerve

The objectives of this project are to determine changes that occur after injury to the sciatic nerve that provides information from the hind limb to the spinal cord and brain.

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

During the presentation it was stated that in humans, brachial nerve plexus injuries pose a bigger problem than those affecting the sciatic nerve; the Committee therefore queried why the current application seeks to work with a sciatic rather than brachial nerve model. The PI explained that compared to sciatic injuries, brachial injuries are very complex and the sciatic nerve model is acceptable for the required proof of principle.

The PI was asked whether the proposed nerve crush injury will be painful for the animal. The PI explained that the crushed nerves will regenerate quickly – within three days, there will be no significant signs of the injury. There is potential for pain during the regeneration process but the animals will be given appropriate analgesia to minimise this. The NACWO confirmed that from previous experience, with appropriate analgesia, the animals show remarkably little sign of discomfort or limitation.

In relation to the sciatic nerve section model, both the sensory and motor nerves will be cut (sectioned) and so the animal will initially experience numbness rather than pain. There is some risk of chewing/nibbling associated with such numbness but this is rare. However, it was felt that the humane endpoints associated with the section model should be clarified. There is likely to be some pain caused as the nerve regenerates and appropriate analgesia will be given to ensure that the animal is comfortable.

The Committee queried whether the animal's footedness (i.e. left or right footed) should be taken into account, for example only carrying out the procedures on a non-dominant limb. The PI responded it was not clear whether rodents have dominant/non-dominant hind limbs but this may be something to look at in the future.

It was noted that the nerve will be cut and the tubing to encourage nerve growth will be inserted straight away, which differs from the human experience of such an injury. The PI explained that peripheral nerves take a long time to die so the likely delay in treating equivalent human injuries does not render this animal model invalid. It was noted

that the longer the delay in humans, the less successful the regeneration. However, it is better from an animal welfare perspective to insert the tubing straight away under the same episode of anaesthesia than to carry out two surgical procedures requiring two separate periods of anaesthesia.

In relation to the nerve section model it was explained that a centimetre of nerve will be removed to create a gap. Again, this is different to the likely human experience of such an injury; in humans the gap is created by shrinkage of the two ends of cut nerve occurring during any movement of the limb post-injury. Calipers will be used to measure the gap. The protocol should be amended to clarify that a length of nerve will be removed rather than a single cut being made. The Committee noted that in the application the length of the gap should be consistent throughout.

Given the multiple steps within the proposed procedures, the Committee asked for some indication of what is likely to happen to a single animal and over what timescale. The PI explained that for the majority of the animals the nerve will be cut, a tube inserted, and this step will be followed by behavioural tests.

In the application, the electrophysiological recordings should be changed to classification 'AC' rather than 'AB'.

Regarding the speed of recovery, the PI explained that it is usually possible to get an electrical impulse between the cut nerve endings within three months. Given this, the Committee queried why it may be necessary to continue the experiments for up to six months. It was explained that to measure myelination and extent of regeneration may require this extended duration; however, this will only be necessary in approximately 10% of the animals. This will be made explicit in the application.

It was noted that in equivalent human injury, regaining motor function is often more important than sensory regeneration; to maximise translationability an assessment of motor control will be made in addition to assessing sensation.

The PI explained that these experiments will mainly use rats rather than mice, with approximately 6 per group and 30-40 per experiment. Some models (including those already used by collaborators) use genetically altered animals, which usually requires the use of mice rather than rats. It was noted that the protocol should be amended where appropriate to clarify whether the animals will be rats or mice and if mice, will be wild type, or genetically altered.

It was queried whether control substances will be used (i.e. 'vehicle controls'). Any vehicle controls and the proposed sham experiments should be explained clearly in the protocols and in part D.

The non-technical summary should be amended to clarify technical terms, for example 'soft mash'. The nature of the proposed nerve injuries (both nerve cut and crush) should also be more clearly explained.

It was explained that the Home Office Inspector is keen to avoid the use of metabolic cages for obtaining urine samples and instead prefers the use of hydrophobic sand in normal animal housing.

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

Responses to the comments provided by the Home Office Inspector have already been incorporated into this application.

Resolved that:

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

15/08-07-2 Application Ref TBA – Evaluation of therapeutics for the treatment of head and neck cancer

The aim of this project is to provide a platform for the identification and validation of novel drugs for the management of head and neck cancer.

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

In the application it is currently stated that surgical resection of tumours will take place on terminally anaesthetised mice. However, this is an error as such resections will occur post-mortem.

The Committee queried the process for measuring tumour size and asked about the level of accuracy with which tumours will be measured. In particular, some concerns were expressed about potential variability given the very low sample sizes. It was explained that the animals will be held by the scruff and calipers will be used to measure the tumour to an accuracy of 0.1 millimetres. The same person will carry out each measurement to reduce variability. It was suggested that measuring the tumour in one dimension is insufficient as some

tumours have substantial depth as well as breadth; it was felt that the measurement should be one of volume rather than area.

The adverse effects stated in the application are more associated with drug treatment than with xenografts; the adverse effects of xenografts should also be included.

The Committee queried whether all animals will be genetically altered and immunocompromised. The PI clarified that this will be the case; such animals are needed to ensure that any effects observed are a direct result of the drug rather than of an immune response.

Further information was requested about the proposed use of the IVIS system, and the PI clarified that if IVIS is used each animal will experience a maximum of 6 imaging sessions at a rate of 2 per week. However, at the current time tumours are manually measured rather than using IVIS as none of the relevant cell lines have the required markers.

It was queried whether all of the drugs will be ones with known effects on cancer cells or whether they will be drugs known to increase the efficacy of other drugs. The PI explained that all of the drugs will be first screened for their effects individually in vitro for their action against cancer cells; the research is not specifically looking at chemo-sensitisers.

In protocol two both irradiation and injection are stated as optional steps; instead, this should be corrected to 'either/or'.

Appropriate pain scoring and the use of analgesia in relation to the insertion of mini-pumps should be included within the application.

As currently written, the number of animals to be used and the timelines of the work were considered unclear. However, it was explained that clarification at this stage is difficult because the numbers and timelines will depend upon the success of the initial stages of the work. The numbers stated in the application are based upon a 3-5 year licence, on the compounds currently being considered by the research group, and on the number of experiments viable with the resources available. The number of animals required will vary with the particular cell line under consideration. Some of the cell lines yield very reproducible results, requiring fewer animals, while others will require larger animal numbers. It was agreed that appropriate wording should be included within the application to explain the reasons why the stated animal numbers are not more specific at this stage and a clearer idea of numbers will be available after pilot studies on cell lines have taken place.

The loss of 20% bodyweight is a standard endpoint for moderate cancer models as the weight of the growing tumour has to be taken into consideration. Body condition scoring will also be used and this should be included within the application.

The main problems associated with tumours are impeded movement and ulceration; endpoints relating to both of these should be included in the application.

The Committee queried how the researchers will decide which cell lines to use for the testing of each drug, and it was explained that there is considerable existing experience to call upon in relation to this. Three particular cell lines, which have known clinical involvement in head and neck cancers will be used. Few experiments using multiple cell lines for any one drug are anticipated; it will only be necessary to get sufficient data for a drug against a single cell line to provide justification for researchers to test efficacy in humans.

As many of the drugs are already used in humans, the Committee asked why the researchers are not going straight to human trials. It was explained that for some drugs that are used in combination, it is difficult or impossible to get information on a single drug from clinical practice and therefore data from animal experiments is still needed. It was also noted that for clinical trials, MTD data are usually required in two species.

It was suggested that the licence should be amended to emphasise that the focus of the work is testing against the xenograft rather than testing dose toxicity.

The Committee agreed that the adverse effects of drugs and the effects of drugs upon tumour cells should be considered in separate experiments rather than together, and that tolerability testing in healthy animals should take place first. The protocols should therefore be amended such that Protocol 1 is concerned with dose setting for novel compounds (without the induction of tumours) and Protocol 2 focuses upon the efficacy of the drugs against cancer cells.

All of the compounds to be tested have shown promise of being efficacious during *in vitro* studies.

Some compounds have been found to have low efficacy on their own, but their efficacy increases when used in combination with another drug. For the purposes of toxicity testing, single drugs would be tested first then any drug combinations. The potential reduction of the required dose of a drug, when used in combination with another which increases its efficacy, should be described as a refinement in the application.

Regarding the non-technical summary, it was suggested that further information should be included about what will happen to the animals. Also, the endpoints should be clarified and the section on the 3Rs should be strengthened.

In the proposed experiments the tumours will be subcutaneous; however, head and neck tumours in humans tend to penetrate quite differently. The Committee therefore queried the appropriateness of a subcutaneous model. It was explained that the PI originally intended to use a model involving tongue tumours in mice. However, it was felt that this was too advanced, and involved too high a cost to the animals, to be a suitable initial model for a relatively inexperienced research group. It is hoped that an orthotopic model will follow on in the future. Additionally, the use of multiple cell lines in the subcutaneous model is a good reflection of the heterogeneity seen in head and neck cancers.

As nude mice will be used, it was queried why irradiation is also necessary. The PI explained that even nude mice are not sufficiently immunosuppressed and that the lowest possible levels of irradiation will be used.

In the non-technical summary, reference is made to multiple analyses being carried out on a single mouse. It should be clarified that this means multiple types of data being obtained from each mouse, rather than reducing the number of animals by using each animal multiple times.

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

Whilst the gold standard for the treatment of head and neck cancers in humans is the administration of cisplatin plus irradiation, there are no facilities within BMSU to carry out localised irradiation.

It was suggested that the PI should consider comparing each drug with cisplatin alone and in combination with cisplatin. Also, as the proposed future clinical trial will be of cisplatin plus one of the new drugs to emerge from this study versus irradiation, the Committee discussed whether this animal work should be carried out at all without the irradiation element. It is understood that work with irradiation is already being carried out with collaborators elsewhere.

The scientific basis for the proposed work should be strengthened in the application, for example the prior *in vitro* work. It was also noted that the focus of the application should be shifted to increasing the efficacy of cisplatin by using additional drugs in combination.

Further information should be included in the application about the pilot studies.

Resolved that:

The revisions discussed above will be made and the revised version of the application will be recirculated to the Committee. Once the Committee is happy with the changes the Chair will recommend that the Establishment Licence Holder submits the application to the Home Office.

15/08-08 Any Other Business

3Rs day

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

ASC letter of 15/06/15

A copy of a recent letter from the ASC, dated 15th June 2015, was circulated to the Committee for information. The purpose of the letter was to share the outputs of a workshop held in November 2014 which explored the associated roles of the ASC and AWERBs.

The ASC will be taking forward the outputs of the workshop and will be back in contact with the University about this in due course.

BERSC PhD student representative

Thanks were given to the current PhD student representative who is standing down from the Committee to write up their PhD. Discussions are underway to identify a new PhD student representative.

Poster presented at NC3Rs/Society of Biology Symposium

It was reported that a member of BERSC recently presented a poster the NC3Rs/Society of Biology Symposium; as an outcome of this they have been invited to join a diet formulation working group which is attempting to make tammoxifen diet more palatable (considered a refinement).

In vivo work carried out in University locations external to BMSU

Further clarification is still needed about the University position on terminal *in vivo* work carried out in approved University locations

external to BMSU, and this will be followed up by the Director of BMSU and the Chair.

15/08-09 Date of Next Meeting

The date of the next meeting is 3rd September 2015.

GLOSSARY

3Rs	Reduction, Refinement and Replacement
AB and AC	Codes used within the Home Office licence application form to describe the anaesthetic status and use of a neuromuscular blocking agent (AB: anaesthesia when the technique is applied but with subsequent recovery; AC: anaesthesia when technique is applied without subsequent recovery)
ASC	Animals in Science Committee
ASPeL	Animals Scientific Procedures eLicensing
AWERB	Animal Welfare and Ethical Review Body
BERSC	Biomedical Ethical Review Sub-Committee
BMSU	Biomedical Services Unit
FOI	Freedom of Information Act
IVIS	In Vivo Imaging System
MTD	Maximum Tolerated Dose
NACWO	Named Animal Care and Welfare Officer
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NVS	Named Veterinary Surgeon
PI	Principal Investigator
TBA	To Be Announced