CONFIDENTIAL MATERIAL

THE UNIVERSITY OF BIRMINGHAM

BIOMEDICAL ETHICAL REVIEW SUB-COMMITTEE (BERSC)

25th September 2014

MINUTES

Present:

14/09-01 Apologies

Apologies were received

14/09-02 Minutes

The minutes of the meeting held on 14th August 2014 were considered by the Committee and were approved.

14/09-03 Matters Arising

There are no matters arising from the previous minutes.

14/09-04 Chairperson's Items

No Chairperson's items were reported.

14/09-05 Verbal Reports from the Director of BMSU and Named Persons

Verbal reports from the Director of BMSU and the Named Persons were circulated prior to the meeting.

14/09-06 Report from the Fast Track Procedure

The fast track procedure is up to date, and there were no further issues to report.

14/09-07 Project Licence Proposals

14/09-07-1 Application Ref TBA – Innate lymphoid cell functions in vivo

The aim of this project is to assess, in vivo, the specific mechanisms by which innate lymphoid cells affect immune responses. The application has already benefited from input from the Home Office Inspector.

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

The Committee was reassured that BMSU has appropriate containment facilities for work using infection models.

It was queried whether sham immunisations will be administered in relation to protocol 1, and the PI confirmed this. The sham immunisations must be explained within part D of the application.

No more than one laparotomy will be performed per animal, and this will be clarified in the application.

Tamoxifen food will be used to induce cre recombinase transgene activity. The PI provided further information about this, explaining that such food has been tolerated well by animals in previous studies. Administration of the food will begin at 5 or 6 weeks of age, and it will be given to both the control and the experimental groups. Any effects due to individual differences in food intake will be assessed using a 'reporter' mouse, which allows the researchers to check that cre has been turned on as intended.

Tamoxifen has some effect upon the immune system, but this is accounted for via the control group. It was queried whether it is appropriate to administer tamoxifen before the immune system is fully developed; the PI explained that by 4 weeks, the animals' immune systems will be fairly robust, and by the start of tamoxifen administration (5/6 weeks) their immune systems should be sufficiently mature. It was noted that the use of tamoxifen food is a refinement because it will avoid the need for daily tamoxifen injections – this should be explained fully in the application.

At the request of the Committee, the PI provided further explanation of the potential benefits of the research in lay terms, and this will be included in the application. It was explained that aim of the research is to identify (and later hopefully manipulate) those molecules on cells which determine whether more or less, or better/worse T cells are produced. By impacting upon T cell production, it should be possible to influence immune system responses.

It was noted that several mechanisms via which welfare costs will be minimised are mentioned in the main body of the application, and these should also be echoed in the NTS.

If the attenuated Listeria monocytogenes strain of mice is likely to have additional health or welfare needs, these should be highlighted as soon as possible to the NACWOs and the NVS. The PI explained that this is unlikely to be an issue, as the strain used will be very specifically targeted – for this reason, it was considered acceptable to include this as a refinement in the NTS, as other such strains are much less robust and would raise additional welfare concerns.

In relation to the Citrobacter experiments, it was queried whether the number of control animals would be reduced after the first few experiments. The PI explained that because Citrobacter has to be made up from scratch for each experiment, the control animals are necessary because it is possible that the bacteria may differ between experiments. The control groups will be kept as small as possible, and the animals will be culled at the earliest possible stage to minimise welfare issues. A firm clinical endpoint for the control mice should be included in the application. It was explained that a number of clinical signs could be monitored in relation to this, such as hunched posture, weight loss, subdued behaviour and faecal bleeding. The PI will consult with other research groups with experience in this model to ensure that the scoring/monitoring systems are robust and appropriate endpoints are defined.

It was queried whether the transplant of mesenteric lymph nodes into the popliteal fat pad could replace the laparotomy model. The PI explained that this was not the case, as the two models are conceptually different.

Under 'reduction' in the NTS, it is stated that the minimum number of mice will be used. The Committee asked for further information about how numbers will be minimised, and the PI explained that tissue from one animal may be used to generate multiple data points, for example obtaining measurements from a number of different locations within the lymph nodes of a single animal. Each experimental group will include 4-5 animals, and each group will be used for 2 experiments.

If cardiac puncture under terminal anaesthesia may be carried out, it should be stated in part D of the application for consistency.

The likely duration of anaesthesia should be clarified within the application.

The Committee discussed the application further after the applicant left the meeting, and praised the PI's presentation. It was suggested that slides from this should be shared with other applicants as an example of best practice.

It was also noted that the reference to bone marrow chimeras should be removed from the application, as this had been included in error.

Resolved that:

With revisions as discussed above, the Chair will recommend that the Establishment Licence Holder submits the application to the Home Office.

14/09-07-2 Application Ref TBA – Arginine methylation and tumourigenesis

The aim of this project is to investigate a particular family of enzymes implicated in cancer development and growth, using cell culture based approaches.

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

It was noted that this is the PI's first project licence, although she has considerable recent experience of relevant animal work. Some start-up funds have been secured for the work, and applications have been submitted for additional funding.

Specific endpoints should be detailed within protocol 2. The researcher clarified that most animals in protocol 2 will be humanely killed by 12 months of age.

Protocols 3 and 5 should be amended to state that animals may be transferred into protocol 6 ("continued use"). The PI clarified that the optional step of implanting a subcutaneous slow release device is included in protocol 6 as an alternative to using hormone pellets, in case there is any difficulty in sourcing the latter.

The PI was advised to consider whether more steps within the protocols should be classed as "optional", to reflect the intended progression of the experiments.

It was noted that p53 knockout mice are more prone to developing tumours, and it may not be possible to palpate all such tumours. For tumours that can't be palpated, endpoints will be assessed via more general signs such as anorexia and piloerection. BMSU already holds p53 knockout mice and has experience in caring for them.

The PI was advised to provide further information in the application about the reasons why this work cannot be carried out in vitro, and to explain that this work is backed by considerable previous in vitro work and work with cell lines.

In relation to protocol 4, the Committee queried how the researchers will select those animals to be used for mating at the end of the experiments. It was explained that this will be on the basis of whether the mammary epithelium reconstitutes after the injection of cells.

The Committee observed that the proposed group sizes are quite large, and queried whether the group sizes are likely to remain constant or to decrease as the study progresses, and whether the total numbers stated on the application are likely to be sufficient. The PI felt that the group sizes are realistic, but it was noted that not all types of experiment will require large group sizes – this will be clarified in the application. The PI agreed to revisit the total numbers and will amend them if necessary. Appropriate statistical advice will be obtained.

The Committee queried how many imaging sessions (and accompanying doses of anaesthesia) will be required per animal. It was explained that this will depend upon the growth rate of the tumours involved, but is likely to be no more than twice a week and no more than 6 in total. Tumours are likely to grow quite rapidly and the animals will be checked daily to identify any side effects.

The application includes a number of specific methods for measuring tumours. It was suggested that this should be decided locally rather than specified in the application, as the most appropriate method will vary depending upon the nature and location of the tumour.

It was felt that weight is not always a good indicator of an animal's condition, particularly in experiments involving tumours, and body condition scoring is used within BMSU for cancer models. Monitoring of foot temperature and colour is also used.

Where relevant, laparotomy should be included as a procedure in the protocols.

The NTS should be checked for typographical errors, and any technical terminology should be replaced or explained.

Resolved that:

Once the revisions noted above have been made, and any changes recommended by the NACWOs have been incorporated, the application will be recirculated to the Committee. Once the Committee is happy with the revised draft, on the basis of Chair's action a recommendation will be made to the Establishment Licence Holder that the application be submitted to the Home Office.

14/09-07-3 <u>Application Ref TBA – Validation of anti-angiogenic and vascular targets in cancer</u>

The aim of this project is to investigate new targets in the blood vessels of cancers to facilitate the development of new treatments.

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

Different models within the application focus upon different markers, and in relation to this the Committee queried whether the intention is to use the models sequentially. The PI explained that this was not the case; rather, tissue arrays will be used as a guide.

The Committee requested further information about the proposed use of immunisation, and the PI explained that immunisation against known markers can be a very effective means of destroying the relevant vasculatures.

The 'moderate' severity banding of this licence reflects the possibility of ulceration at tumour sites. It was noted that any animals showing ulceration would be humanely killed.

Some of the proposed work is new to the PI and to BMSU, and the PI will obtain advice and learn from the experience of others who are already carrying out such work at other institutions.

The Committee queried how the PI will monitor the health of mice that may develop spontaneous tumours. The PI explained that appropriate endpoints and clinical scoring would be used, and these will vary between the proposed models. A marker will be used with all implanted cells, such that they can be monitored using the IVIS system.

The PI was asked to explain the proposed sponge assay, which will be one of the final assays to be carried out for angiogenesis. A small piece of sponge will be implanted into the mouse, and blood vessels will then grow into the sponge. This is one of the least invasive assays possible, with the fewest possible welfare implications.

There are many options listed within the licence application, and it was queried whether the protocols submitted to funding bodies were more specific than this. The PI agreed that the current licence application is much broader than his funding applications, in an effort to cover all possible work and to avoid delays relating to amendments at a later stage.

The Committee queried the use of orthotopic models rather than transgenic models, as in some cases the former raise greater ethical and animal welfare concerns than the latter. The PI explained that in relation to the proposed work, there are problems with some of the relevant transgenic strains, which are not robust. Also, the proposed

implantation model gives 100% tumour development meaning that fewer animals are required than would be for a transgenic model.

Both IVIS and ultrasound imaging are mentioned in the application, and the Committee queried how the researcher will decide which is most appropriate. It was explained that ultrasound could be used to measure blood flow, but BMSU does not currently have the equipment necessary for this. IVIS imaging will be used to measure the size and metastasis of tumours, and the anaesthesia used is likely to be isoflurane. Callipers will be used for measuring tumours within the skin, whereas IVIS imaging is appropriate if a tumour is within an internal organ.

The PI clarified that IVIS imaging is likely to be carried out weekly, but the animals will be monitored daily by staff within BMSU.

In relation to the last sentence of the 'replacement' section of the NTS, it was suggested that examples should be given of alternatives developed during the PI's previous work. In relation to 'refinement', it was felt that the focus should move away from explaining the choice of models used, and the section should instead explain how the models are the best currently available, and should indicate the scope and appetite for further refinement.

The Committee and the PI discussed the possibility that the current licence application may have to be revised to make it more focused, and to remove any models which are not considered essential, as it will otherwise be difficult to justify to the Home Office. Alternatively, the PI may choose to split the current application into several separate licences. It was strongly suggested that the application as it stands should be revised to include clear targets and justification for each of the models to be included, preferably as a table. The PI agreed to consider removing some of the models, for example the bladder and lung models, for which no funding has been obtained to date and in which there is no experience at BMSU. The PI was reassured that if amendments are required, delays are less likely than they were in the past and amendments are now being processed much more quickly.

The NTS will require further work, as given the complexity of the application it is currently very broad.

The licence application has yet to be sent to the Home Office Inspector for comment.

Resolved that:

It was agreed that once the application has been revised in line with the comments above, and has benefited from the comments of the Home Office Inspector, it should be recirculated to the Committee for further consideration.

As a general observation, it was agreed that licences would benefit from some method of non-invasive imaging other than IVIS, for example small animal MRI scanning would represent a considerable refinement. It was suggested that this may be possible via collaboration with a nearby institution.

14/09-07-4 Application Ref TBA – Study & treatment in model of liver transplant

The aims of this project are i). to set up a new model which is similar to the situation found during Donation after Cardiac Death (DCD) liver transplantation, and ii). to test mesenchymal stromal cells (MSC) in this model to establish if they reduce the extent of liver damage.

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

The PI was asked about the duration of the proposed surgery, and he explained that it will require a maximum of around 2 hours of anaesthesia. Body temperature will be carefully monitored during surgery. It is likely that isoflurane will be the anaesthetic used, and in terms of analgesia, opiates will be used rather than NSAIDs.

It was explained that the surgeon who will carry out the work has previous relevant experience in non-recovery animal work, and has surgical experience with human patients. This will be made clear in the application.

In protocol 2, both cell infusion steps are currently 'optional'. It was agreed that they cannot both be optional, and the control arm of the study should be explained in section D.

Further justification for the sham procedures should be provided in the application.

In relation to both protocols, the compulsory step of proliferation assessment should be removed and instead this should be explained in part D of the application.

The PI confirmed that he has worked closely with a statistician in relation to the power calculations described in the application.

It was queried whether the proposed infusion of MSCs will require a second laparotomy, and the PI reassured the Committee that this would not be the case, as all work will take place during a single operation. This should be clarified within the application.

Regarding the anaesthesia required for imaging, the PI clarified that a maximum of 2/3 sessions will be required.

It was explained that no liver-specific endpoints (e.g. jaundice) are anticipated, as the liver injury will be relatively mild.

The reference to intravital microscopy has been included in error, and should be removed from the application.

The PI was advised to revisit parts D and E of the application, to ensure that they are consistent with information detailed elsewhere.

Within the NTS, the references to schedule 1 methods of killing and non-schedule methods should be clarified to avoid confusion.

The PI was advised to amend the section on 'refinement' in the NTS to demonstrate how the models are the best possible at the current time, and to show a willingness to continue to refine the work going forwards.

The references to appendices should be removed, as they have been included in error.

The licence application has yet to be sent to the Home Office Inspector for comment.

If human MSCs will be used, immunodeficient mice will be required. This will be clarified in the application.

It was agreed that the basis for anticipating few adverse effects should be explained in the application, and more information should be provided about the ongoing monitoring of the animals.

Resolved that:

Subject to the revisions noted above, a recommendation will be made to the Establishment Licence Holder that the application be submitted to the Home Office.

14/09-08 Any Other Business

Freedom of Information requests

Two more Freedom of Information requests relating to animal experimentation have recently been received. It has been possible to resolve these by referral to the new externally facing BMSU webpages, which are proving very useful.

14/09-09 Date of Next Meeting

The date of the next meeting is 13th November 2014.

GLOSSARY

BERSC	Biomedical Ethical Review Sub-Committee
BMSU	Biomedical Services Unit
DCD	Donation after Cardiac Death
IVIS	In Vitro Imaging System
MSC	Mesenchymal Stromal Cells
MRI	Magnetic Resonance Imaging
NACWO	Named Animal Care and Welfare Officer
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NSAID	Non Steroidal Anti Inflammatory Drug
NTS	Non Technical Summary
NVS	Named Veterinary Surgeon
PI	Principal Investigator
TBA	To Be Announced