The University of Manchester

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 21 July 2022

Present: [redacted]

Apologies: [redacted]

In attendance: [redacted]

1. Minutes

Agreed: That the minutes of the meeting held on 23 June 2022 were approved subject to a correction of attendance. The Chair did not attend and the meeting was taken by the Deputy Chair.

2. Update on applications outstanding from previous meetings and upcoming Project Licence applications

2.1. The committee were provided with a document showing the status of applications considered previously and those pencilled in for future meetings. A verbal update to the document was given.

3. NACWO and Directors report

3.1. No comments were made on the report submitted.

4. NVS report

4.1. Caecal torsion was discussed and how the use of appropriate enrichment in the cages appears to have reduced the incidence.

4.2. The difference in allowed weight loss on licences was raised and if AWERB needed to have a more consistent view on this. In addition, the point was raised that weight loss on its own may not be a sufficient indicator for animal health and that instead a panel of measurements/observations should be considered and that the cut-offs should be evidence based. The possibility of having an NC3Rs project to gather evidence on this topic was discussed. This matter will be covered in an away day so that there is more time to discuss this.

5. Standard Conditions 18s and non-compliances

5.1. The committee were provided with a table of reports submitted to ASRU along with the reports for each incident. Since submission of the paperwork, ASRU have closed two cases:

5.1.1; ASRU_University of Manchester_[redacted]
ASRU have requested reconfirmation of competency for oral gavage. A discussion took place regarding the gavage tubes, specifically the differences between rigid and flexible tubes. The rigid ones are reused after autoclaving and some groups prefer using these and have low incidence of adverse effects. Other groups prefer the flexible tubes as they are less likely to pierce the oesophagus but animals can bite through them. The flexible tubes are disposed of after one use.

5.1.2; ASRU_UoManchester_[redacted]
The Home Office Inspector has requested a rewording of the licence to ensure it is clear what the weight loss for the humane end point is.

6. 3Rs AWERB subgroup report

6.1. No comments were made on the minutes and reviews submitted.

7. Applications for New Project Licences

7.1. [redacted], Ex Vivo Gene Therapy for Diseases of Skeletal Muscle

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

Interviewed: [redacted].

Discussed:
- There were a lot of pre-submitted comments from reviewers but these were about how the licence was written and not about ethical or welfare concerns.
- How to deal with NTSs that are too technical. Suggestions included a red flag system at pre-AWERB meetings whereby lay members are

contacted if the NTS is thought to be too technical. This would allow it to be updated prior to the AWERB meeting. This comment was not specific to this licence application but the process in general. The pre-AWERB meeting would need to be far enough in advance of the AWERB meeting for this to be possible. The topic has been added to the list of items to discuss at an away day.

- The wording for ear punching was raised. [redacted] explained that this is standing wording from ASRU. This is also the case for the general constraints section; ASPeL includes details which cannot be altered by the licence applicant.

Revisions:

- Project title - The project title could be changed to more accurately reflect the work. A suggestion would be "In vivo gene therapy for diseases of skeletal muscle".
- Page 10 - The funding account is not specific enough; please include amounts and clarify if the dates are end dates.
- Page 18 – please clarify if the disease model animals are homozygous or heterozygous.
- Page 18 - Is it really the case that all strains have no or a mild severity phenotype? Is breeding controlled in such a way that even mdx mice develop no signs? Protocol 1 mentions AGED so assume there should be a phenotype and therefore perhaps this needs clarification if it is indeed at worst mild (AND some have altered immune system - not sure this is entirely internally consistent).
- Page 20 – Please explain what an NSG background is.
- Page 25 – please clarify if the cell being transplanted from DMD mutant mice, and corrected, or from another source.
- Page 27 - Step 1, are these mice adults only or could be juvenile or aged?
- Page 28 – Please include volumes in Step 2. Please ensure the dosing regimes are more detailed throughout the whole licence.
- Page 32 - step 5 has both mandatory and optional components listed under an 'optional' heading. Please correct this.
- Page 36 – "How will you determine group sizes?" A worked example for sample size determination should be included in this section and for each protocol.
- Page 37 – please can you clarify the disconnect between manifestation between 6 and 8 months and maintaining animals for ~ 18 months as discussed in the meeting.
- Page 42 – please check with the Named Persons in the BSF if Step 2 is required in the licence.
- Page 46 – please provide incidence rate not a number for proportion developing tumours. As written this could be taken as 100% incidence.
- Page 54 – Please can you explain the terms e11.5 and P60 the first time they are mentioned.
- A number of comments were made regarding your Non-Technical Summary which are listed below. **Please update your NTS based on the comments and send it to the following lay members for their review** [redacted]

[redacted]

- Page 2 and 3 - This is the Non-Technical Summary, but the material presented varies between the clear, as for example in the "What's the aim?" paragraph, and the obscure, because it is full of unexplained technical language and acronyms, as for example in the "Why is it important?" paragraph. This the case throughout the NTS, and I have listed some examples below. But overall it does not make easy reading for the non-technical reader - and it should. It would appear that a good deal of the "Why is it important?" paragraph has been cut-&-pasted, complete with reference listings at lines 3 and 7. While some terms are explained, others are not, and the non-technical reader is quickly lost. Examples in the paragraph beginning "Congenital muscle diseases" include: exon skipping, PTC124, progenitor cells, HLA-matched donors, the expression of dystrophin.
- Page 3 – The aims of the project could be addressed more directly - I didn't understand that testing of an improved ex vivo therapy, development of artificial muscle, tumour evaluation or cell lineage tracing were being examined until I'd read the protocols.
- Page 3 - Why is it important: causing, replace with causes
- Page 3 - Why is it important: first paragraph, should specify that transplantation will be into skeletal muscle, and in mice.
- Page 4 - "dystrophic pups are counter selected" and the subsequent sentence need explaining: the material makes sense, but is not easily accessible to the Non-Technical reader.
- Page 4 – please use the term suffering rather than sufferance. Also, please get advice from the Named Persons in the BSF if it is accurate to state that animals experience no suffering.
- Page 5 - Typically, what will be done to an animal used in your project? – drug should be drugs
- Page 5 – Please can you clarify for a lay reader the following in the NTS: "intra-peritoneally", "biomaterials may be combined with cells" - what biomaterials?, " de novo", "dehiscence".
- Page 5 - "Replacement" section: the first two paragraphs simply repeat the "Aims" material on page 2. Where does this material belong?
- Page 6 - cell cultures and organ cultures - these need explaining: what is the difference?
- Page 6 - "How have you estimated the numbers?" paragraphs - the first paragraph is indigestible for the lay reader: it has too much detail, all of which makes sense on careful re-reading - but this is the NTS. It could be much less detailed and more informative. In the second paragraph, please explain "homozygosity". Also can project be renamed aims. Both of these aims should be referred to in the Aims section of the document.
- Page 6 and 7 - "What steps" and "what measures" paragraphs are full of unexplained terms, and read as if they have been copied from elsewhere rather than written for the non-technical reader.
- Page 7 - Refinement: please explain "anthocyanins".

- Page 12 – the first paragraph of the new knowledge section would fit better in the current state of scientific knowledge section.

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], Development, Refinement & Validation of Small Animal Imaging

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

Interviewed: [redacted]

Discussed:
- The licence has progressed from a service licence to one that includes protocols more alike conventional research licences therefore it requires input from the statistician.
- Animals are singly housed after surgery until after recovery to avoid damage to surgical areas.

Revisions:
- Please obtain input from the statistician for this licence, specifically in relation to Protocols 3 and 4.
- The numbers are not consistent across the paperwork. The number on the Cat A form is 900 however the number on page 8 is 850 and if the numbers on the Protocols are added up they come to 950 for each species. Please ensure the numbers are consistent.
- Please ensure more information is included regarding the inclusion of rats as discussed in the meeting.
- Page 26 - Given some of the possible uses given in the background e.g hypertensive rats, dementia models, is it really the case that step 3 will have nothing more than a mild severity level?
- Page 32 - Please describe how new imaging mini-projects under protocol 2 will be selected, prioritised, and how the decision would be made to terminate them.
- Page 33 - Please clarify if you will be measuring oxygen at the same time as administering CO_2.
- Page 33 – As discussed in the meeting, 10% CO_2 is not supported by AWERB and a lower level should be administered. Please discuss with the Named Persons in the BSF an appropriate level. 7% was discussed in the meeting.
- Page 33 – please include how often the substances will be administered into the same animal.
- Page 34 – as discussed in the meeting, please include information based on evidence from the imaging unit on adverse effects expected from imaging. The percentage given currently, (~1 in 1000), appears to be very low.
- Page 37 - All data will be made publicly available after a period of 18 months. Please include details of how this would be done.
- Page 39 - please consider frequency rather than using rare as an indicator of how many animals may undergo a step or not.
- Page 44 – if possible within the ASPeL system, please can you make it clearer that the superscripts in the table relate to the references given.

- Page 46 - In "What are the human endpoints for this study" there is a disconnect between S1 and the preceding sentence. The sentence needs expanding to make sure that the correct meaning is given.
- Page 46 - the maximum frequency of administering substances (max vols are given) should be indicated.
- Page 46 - Under "What are the likely adverse effects" and "How will you monitor……" it would be useful to know what the total loss of animals might be.
- Page 58 – It would be useful to include some more information regarding the health and safety for the human carrying out this work here.
- Page 59 - correct CUT for GUT
- Page 68 - Typo: "We may also repeat experiments to confirm important findings that in separate cohorts."
- A number of comments were made regarding your Non-Technical Summary which are listed below. **Please update your NTS based on the comments and send it to the following lay members for their review** [redacted]
    - Please use humanely killed not culled in the Non-Technical Summary.
    - Page 3 - Why is it important to undertake this work? This section would be made shorter.
    - Page 6 - Up to this page, the NTS does more or less exactly what it should, namely explain to the non-technical reader what will happen to the animals used in this research, and why. On page 6, jargon appears, and the paragraph beginning "we will induce anaesthesia" uses a number of non-explained terms which need either explaining or, in some instances, omitting altogether. As an example, much of the details in the following isn't necessary for the reader to understand what is happening to the animals involved: "animals will be injected intravenously, intrathecally, intracranially or intracerebroventricularly with contrast agents/dyes (e.g. gadolinium-chelate, nanoparticle, MPIO-mAbs, deuterium-labelled compound, fluorescent dye, radioactive tracer, or any agent that is given to enhance image contrast) or compounds to inhibit or promote certain physiological functions (e.g. glucose transporter inhibitor, water channel inhibitor)".
    - Page 6 – please use % incidence rather than 'rare' or 'very rare' as the latter are not quantifiable to the reader.
    - Page 7 - The second and third paragraphs also include terms that need either explaining (e.g. "culled immediately via S1"), or omitting (e.g. e.g."Intrathecal, intra-cisterna magna, intracerebral, and intra-cerebroventricular"). The labels for the various genetically-modified strains of mice (e.g. 5xFAD) don't explain anything to the non-technical reader, who is also unlikely to understand "amyloid angiopathy (e.g. APP/PS1)".
    - Page 8 - "Which non-animal alternatives" paragraph. Please explain for a non-technical reader what "phantom" refers to as used in this

paragraph, and explained what "3D printed biophantoms" are. In the "why were they not suitable" paragraph, "the molecules whole-body pharmacokinetics and interactions with other receptors/binding sites" needs a little unpacking, (as well as an apostrophe...).

- Page 9 - "We will ensure that the minimum number of animals are used to measure the expected effect size by performing power calculations prior to undertaking any experiment." I would remove this statement from P9 as it does not apply to protocol 2 at all, and only partially to protocols 3&4.

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], Factors Regulating the Skin Immune System in Health & Disease

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

Interviewed: [redacted]

Discussed:

- The statistician is satisfied with the numbers on the licence and will sign the form to this effect.
- The skin and gut microbiome may be involved but the studies do not investigate this as yet.

Revisions:

- As discussed in the meeting, please include details of how many animals will undergo anaesthesia for 7-10 days for consecutive anaesthesia with interventions as this is a long time. The length of time the animals will be anaesthetised for should also be included.
- Page 19 – we suggest you remove the phrase 'to at least home office minimum'. You state “aseptically” which is sufficient as a statement.
- Page 27 – “How will you determine group sizes?” A worked sample size calculation would be helpful as given on page 38.
- Page 32 - The first sentence in "Depletion of cells by administration of Diphtheria toxin" does not make sense. Is something missing?
- Page 32 - volumes and routes paragraph to refer to frequencies in preceding paragraphs could be specifically stated. This paragraph is then contradicted by the one numbered 1 where up to 7 injections can be given. This section needs tidying up so that it is clear what the maximum volumes, routes and frequencies are. This is also the case for Protocols 3 and 4.
- Page 43 - In the first paragraph it should read "…..while they are still alive, by measuring……"
- A number of comments were made regarding your Non-Technical Summary which are listed below. Please update your NTS based on the comments and send it to the following lay members for their review [redacted]

- Page 4 - Rather than say: 'The project will benefit pharmaceutical companies', please say the project will benefit 'drug developers at pharmaceutical companies' to avoid anything being misconstrued.
- Page 6 - This is a very clear NTS, and it was only on page 6 that I came across terms that need explanation: e.g. "Skin inflammation may also be induced by treatment with immune modulating agents such as cytokines or chemokines with or without tape stripping", "piloerection, folliculitis or epidermal necrosis"
- Page 6 – please include where typically on the body are these topical agents are administered.
- Page 8 - "Which non-animal alternatives" paragraph" has a number of non-explained technical terms.
- Page 8 and 9 - I think you are giving more information here than is helpful - in particular I found the paragraphs beginning "In order to examine" and "The most efficient breeding strategy" unhelpfully complex.
- Page 9 – The section "What measures, apart from good experimental design" could be more succinct.
- Page 9 - Could this section be shortened with less scientific information. Please consider describing the principles rather than the experimental details.

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. Joint RSPCA/LASA/LAVA/IAT meeting on induction and training for AWERB members

A reminder was given regarding the meeting in Birmingham. The Director of Research and Business Engagement would support costs for lay members to attend the meeting.

8.2. ASRU audit

The report is expected by the end of the month. It will be shared with AWERB once received.

8.3. [redacted]

[redacted] is stepping down after the away day in September. The Chair thanked [redacted] for his valuable input over the years he was served.

The next meeting will be on 22 September 2022 at 10am-12.30pm.

Dates of meetings for the 2022/2023 academic year are:
22 September 2022
20 October 2022
17 November 2022

15 December 2022
26 January 2023
23 February 2023
23 March 2023
27 April 2023
25 May 2023
22 June 2023
20 July 2023
August break