MANCHESTER 1824
The University of Manchester

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 17 November 2022

Present: [redacted]

Apologies: [redacted]

In attendance: [redacted] (trainee NVS)

1. Minutes

Noted:
- Section 2.2 on page 3 of the minutes of the meeting held on 20 October 2022 should read 'understanding' not 'understand'.
- Section 7 – a correction was needed regarding suspension of access to the unit, rather than the incorrectly stated licence being suspended.

Reported: The incident 'ASRU_University of Manchester[redacted]_sc18' is not being progressed any further.

2. Applications for New Project Licences

2.1. [redacted], In vivo studies of pathways and cells eliciting cancer immunity following detection of damage and microbes

Considered: A completed AWERB form and PPL application

Interviewed: [redacted]

Discussed:
- The application cannot proceed in its current format. The application requires revisions, input from the statistician, input from a lay

member for your NTS, and more detailed discussions with staff from the germ free facility.
- You must attend a pre-AWERB meeting with BSF staff so that the application can be in revised to their satisfaction before it comes back to a full AWERB meeting.

Revisions: It was explained to the applicant that the committee had provided comments to the Secretariat prior to the meeting and while some would be discussed in the meeting, the list below includes all the comments whether they were raised in the meeting or not.

- With regards to publishing of negative data on the Cat A form. It is important that *all* results be published; their benefit to "a big part of the scientific community" is something that can only be judged *by* that community; it's not for the researcher to stipulate in advance - and, of course, "big" is a very vague term. Likewise, it is not clear why journal guidelines should be relevant when it comes to publishing raw data; one might wonder whether any journal does or should have that kind of veto power, and if any journal does claim it, whether researchers perhaps ought simply to find another journal. Note that raw data could simply be hosted on a UoM server which we would encourage.
- The numbers on the Cat A form differ from that on the Home Office form. Please revise to make it consistent and clear as the Cat A form is the one that receives signatures from the Named Persons.
- The information in the protocols requires revision and some of the points are listed below however significant input and guidance is required from the BSF staff.
- "Why are you proposing this severity category?" You need to consider all of the steps in each Protocol and cumulative effects of all the treatments/surgeries/injections/metastases etc. and not only the severity from superficial tumours.
- Page 13 - What support does application have - Could add the support provided by UoM for maintenance of germ-free animals.
- Page 14 – Please include which protocols will be at UoM.
- Page 14 – This should be additional establishment not secondary. The additional establishment should appear on Page 1.
- Page 14 - Who will be responsible for supervising your work at this additional establishment? This should be Vicky Taylor.
- Page 15 - this could be better explained. To transport GA animals from the breeding establishment to the germ-free facility? Unsure how this can happen. The GA animals will not be created germ-free.
- Page 20 - Will all procedures be undertaken in germ-free animals? How will germ-free status be maintained?
- Page 25 - Proposed severity - does a superficial tumour with a scab constitute an abscess? What is the algorithm for managing these?
- Page 24, Page 49 and all further protocols. Move "Notes-pictures of “tumour condition” to assist identification of the various grades will be used when available. Post-operational transient display of weight loss and clinical signs (i.e. inappetence / dehydration)." under the monitoring and control sections in relevant steps of this protocol.
- Page 27 – Please remove the [redacted] reference.

- Page 30 - Germ-free mice are provided with additional enrichment materials which reduces the likely incidence of gut torsions.
- Page 30 - Protocol 1, Step1:
    - Ulcerated tumours:
    - All ulcers initially go through the "wet ulcerated" stage before they form a scab, just the matter of when you look at the animal. No treatment (i.e. clay or emollient) is usually effective once the tumour is ulcerated. If you really need to keep animals with ulcerated tumours, I would suggest making it very clear and adding very good justification for that and the limits of the ulceration (e.g. if ulcer is more than 10% of the tumour or = HEP). If there is no particular need to keep animals with ulcerated tumours, I would state that in the application and make any ulceration a HEP.
    - HEPs:
    - Are you planning to re-suture open surgical wounds before 48h after surgery? In the case of open surgical wounds, the implanted tumour piece is usually lost.
    - You will be injecting tumour cells IV and IP, what are the expected adverse effects of those tumours? Internal tumours? Metastases? How will you monitor and control those? Palpation? Monitoring breathing patterns?
    - Metastases for other types of tumours - SC and tumour pieces injections? Monitoring? HEPs?
    - "Pain not controlled by analgesia" - will you be providing analgesia for the surgical implantation of the tumour pieces?
- Page 31 - Will primary tumours be removed from germ-free mice too?
- Page 31/32 - Protocol 1, Step 3:
    - Will you be removing only superficial sc tumours? Risks and adverse effects would be very different when removing a sc tumour vs trying to remove tumours of internal organs.
    - Clay shouldn't be needed for surgical wounds.
    - Surgical wound break down time limit HEP? Would you be attempting re-suture?
    - Define "persistent" bleeding from biopsy site? Surgical resection site shouldn't be bleeding at all.
    - You mention that tumour and metastases will be HEP. How will you be monitoring for those? Especially for internal tumours/mets? Will you be using any imaging?
- Page 32 - Humane endpoint - how long is persistent bleeding.
- Page 32 - Protocol 1, Step 4:
    - HEP:
    - How will you measure "inappetence", especially in group housed animals?
    - "Display of weight loss and signs of toxicity as described above (i.e. inappetence / dehydration, decreased mobility) with no obvious signs of recovery within 8h." - careful with this statement. This both means that you will be assessing and weighing your animals every 8h (otherwise how do you know if they are recovering or not) and also culling animals that don't

gain any weight within any 8h period, which is very likely with irradiation.
    - "Irradiated gut can result in malabsorption with associated weight loss and the growth of microorganisms resulting in enteritis." - I would suggest moving this sentence from HEP to the expected adverse effects section and putting signs of enteritis in your HEP.
- Page 32 - Step 4 - will GF mice be irradiated?
- Page 33 - Mitigation for irradiation, Is acidified water still used? UoM has phased this out due to more refined procedures being available (sterile water) and mice find the water unpalatable. Is there a more refined process?
- Page 33 - Irradiated gut can result in malabsorption with associated weight loss and the growth of microorganisms resulting in enteritis. This is not a humane endpoint and requires revision.
- Page 34 - Protocol 1, Step 5:
    - "c) Physical restraint in a jig (AB/AC)." - why do you need to restrain them if they will be under anaesthesia?
    - "d) Endoscopic imaging of the colon (AB)."- this procedure is quite invasive and requires further considerations (e.g. flushing out faeces, potential pain and adverse effects re perforation, rectal prolapse etc.) and adding adverse effects and HEPs to the step.
    - "g) Mice will be exposed to imaging sessions no more than twice per week (AB) or 20 per lifetime
    - (AB)." - this should be a separate g) step. Also, why are they all AB if some of the imaging steps are AA?
    - Need to clarify which imaging sessions can be combined with which frequency and intervals between them. Having colon endoscopy twice per week will be very different from having 2 ultrasounds per week.
- Page 34-36 and other similar steps in other protocols
    - Protocol 1, Step 6:
    - "a) In situ photoconversion. The animals will be anesthetized following surgical exposure of tissues
    - will be illuminated once per lifetime with light of specific wavelength (AB/AC)." - will only intestinal cells be illuminated? Expected adverse effects are only for GI cells.
    - "b) Physical restraint in a jig on up to 3 times (AB/AC)." - why restraint needed of anaesthetised animals?
    - "c) Whole body imaging or non-invasive intravital microscopy on one or more occasions (AB/AC);
    - and/or
    - d) Once only intravital microscopy following surgical exposure of tissues (AC)." - would those 2 not be covered by step 5 in this protocol?
    - "In the period during or following operation animals are susceptible to infections." - will you be entering GI tract? Otherwise, why expected adverse effects are different from other surgeries in this protocol?

    - "Animals will be assessed for fitness before being subjected to anaesthesia and aseptic conditions using LASA guidelines (according to Hoogstraten-Miller SL, et al Curr Protocol Immunol, 2008, Chapter:.12-.14." - why this is not mentioned in other steps using surgical techniques? Need more standardisation throughout the PPL.
    - "Mice can develop post-operative ileus if the illuminated tissue is the intestine" - how will you monitor for that? How often? For how long?
    - "Display of weight loss and signs of toxicity as described above (i.e. inappetence / dehydration) with no obvious signs of recovery within 24h." - it is not described above.
- Page 34 - Step 5 - will imaging of GF mice be terminal?
- Page 35 - Step 6 - In GF mice?
- Page 35 - How will the skin be sutured "sufficiently"?
- p36-37 + other similar steps in other protocols
    - Protocol 1, Step 7:
    - What are the max volumes and max number of administration per each route of administration? Is there a table describing that in the PPL?
    - "Administration of therapeutic agents on one or more occasions by one of the following possible routes." - can all those routes be combined and used in one animal? What are the max volume per each route?
    - Need to add adverse effects/monitoring/HEP for surgical implantation of minipumps. Are you planning to implant minipumps once only in each animal?
    - "a) Oral gavage either once or twice per day for up to 3 months" - this is a lot of OGs, so the cumulative adverse effects should be considered. Same with numerous injections.
- Page 36 and 84 - In step 7 and multiple times throughout the application where "b" is given twice and the second is followed by "c".
- Page 37 and 38 - Step 7 - Are there any adverse effects from surgery for pin-port or osmotic minipump insertion?
- p37-38 + other similar steps in other protocols
    - Protocol 1, Step 8:
    - See above re max volumes, max numbers, cumulative severity and adverse effects of numerous administrations via OG and injections in one animal.
    - "The injections doses will be kept to the minimum possible and the appropriate volume per route of administration will be used following the guidelines of JWG: Morton et al, 2001, Laboratory Animals, 35, 1-41." - I suggest inserting a table specifying max volumes and numbers of administrations per route.
    - "intratracheally" - should be AB code.
    - Need to add adverse effects/monitoring/HEP for surgical implantation of minipumps. Are you planning to implant minipumps once only in each animal?
- p38-39 + other similar steps in other protocols
    - Protocol 1, Step 9:

    - "b) Orally, by gavage or in feed or drinking water (AA).
    - c) Rectal administration (AB)
    - b) Orally, by gavage or in feed or drinking water (AA)." - repetitions.
    - Intratracheal should be AB code
    - "Substances fro monitoring…" on p38 - correct to "for"
    - See above about frequencies and numbers of administration.
    - "If a mini-pump is used there may be a transient discomfort, but no lasting harm. " - any other surgical complications? Wound break down?
    - "Display of weight loss and signs of toxicity (i.e. inappetence / dehydration and reduced activity)
    - with no obvious signs of recovery within 24h." - careful with this statement as mice treated with Tamoxifen tend to show weight loss for longer that 24h before they start recovering.
    - "Surgical wounds that open post-surgery beyond a 48h timeframe." - what if they open in the first 48h?
- Page 39 - At multiple places in the application the authors use "closed weighed". Please explain what this is.
- Page 40 - Step 10 – there are no adverse effects of custom diets, therefore please remove those listed.
- p41-42 + other similar steps in other protocols
    - Protocol 1, Step 12:
    - "If tumour regression occurs, mice might be given an injection of tumour cells…" - does this mean complete or partial regression? Will some primary tumour be present?
    - "Certain tumours and particularly those grown subcutaneously or intradermally may show signs of reddening or scabbing" - scabbing usually means ulceration, need to account for that in your expected adverse effects. See comments to step 1 (p30) above re ulcers.
    - HEP for the size of the tumours needed. How and how often will you measure tumours? Will you use imaging to monitor for internal tumours?
    - With any tumours, when you calculate body weight loss, you need to account for the weight of the growing tumour/tumours. How will you do that (especially with internal tumours)?
- Page 42 - step 12 -This is much more in depth than the initial tumour implantations step. Will challenge and re-challenge be dealt with in similar ways?
- Page 43 - Animal experience - this is ambiguous. Do you mean Incubator at UoM? What about germ-free animals? Will the primary tumour be resected?
- p43-44 "Protocol 1 - Animal Experience
    - On p4 in NTS you mentioned ""Typically, in any experiment mice will experience up to 3 procedures for a period of up to 12 months."" Given the proposed combination and frequencies of treatments/injections/surgeries described in Protocol 1, those would exceed 3 procedures in 12 months.
    - What will be the max amount of surgeries one mouse will experience throughout the life?

    - What will be the max number of tumours one mouse could bear in its lifetime (excluding metastases)?
    - "Mice will be bred at the Incubator and at around 3-4 weeks of age (immediately after weaning) will be transported the short distance to the research site."" - how will you maintain animal germ-free during transport and during all the experimental manipulations?
    - Given the cumulative effects of the tumours, metastases, treatments, surgeries, I would say that Moderate severity will be experience by almost all animals and not only 10% as described at the beginning of the protocol 1. "
- Page 46 and 71 - "Careful thought will be given….." appears several times in the application. Thought about experimental design should take place prior to submission of the application.
- Page 46 - Determining group sizes:
    - What is your experimental unit? When you inject tumour cells on both sides of a mouse, will you always be able to treat those two tumours differently? If you group house animals and treat them with an altered diet, will you consider the cage as your experimental unit?
- Page 47 - Protocol justification - The Action Plan as written does not give the full specific justification. Please revise this section.
- Page 52 - Protocol 2, step 1, I can't see the actual mechanical challenge stated.
- Page 53 - Will you be using Tumour prone strains to induce further tumours? Meaning that MMTV-PyVT mice will both develop mammary tumours and will be induced with further tumours with injection/colitis/tamoxifen? You might need a separate protocol just for the tumour prone strains.
- Page 53 - " Each of the methods a,b, c or d will be performed individually." - there is no step d)
- Page 53 – You need to add expected adverse effects for each of the tumour type (colorectal - obstruction, bleeding etc; mammary; skin etc.), metastases?
- Page 54 - step 2 - tamoxifen transgene induction – is this only in GA animals? All GF mice are wild type.
- Page 54 - Will GF mice receive DSS as they are prone to diarrhoea anyway?
- Page 56 - How long is persistent anaemia?
- Page 67 – The scale mentioned is not provided. Please include this.
- Page 69 - "Mice will have tumour arising spontaneously following administration of carcinogens and their growth will be monitored by use of callipers or ultrasound." - mention ultrasound monitoring in the tumour step itself.
- Page 69 - In the sentence "Finally, some animals…." there is no alternative to the "either" which ought to be present.
- Page 74 - Review the proportion of animals that will experience moderate severity based on the cumulative effects of all the steps in the protocol. Review "Why are you proposing this severity category?" section (see above).

- Page 77 - Protocol 3, step 1, Do germ-free animals need any additional care/controls being already in a colonic compromised state?
- Page 78 - "Diarrhoea is expected to be seen in some of the mice and is not considered an adverse effect in this protocol as it is one of the main symptoms of colitis." - it is still an adverse effect even if expected. "Depending on the mouse availability, male mice of approximately 8 weeks old will be preferentially used to minimise the adverse effects as they usually exhibit reduced symptoms of colitis following DSS administration." - please review this statement as usually female mice show less clinical signs (https://pubmed.ncbi.nlm.nih.gov/25962374/).
- Page 78 - "If a mouse displays any signs of pain-related behaviours analgesics will be provided in a form of gel." - I would not restrict analgesia type to just gel. HEPs - Diarrhoea and dehydration HEPs?
- Page 80 - When and how will analgesia be provided?
- Page 89 – please include the site of implantation of the mini pump.
- Page 90 – Please clarify why immunisation would cause pain.
- Page 98 - Which adjuvant will you be using and what are the expected adverse effects of those? Are you expecting any local inflammation?
- p98 vs p121 - Immunisation step. The same step, but control of adverse effects are different in Protocol 4 from Protocol 5.
- Page 100 – please clarify what the curves are you mention.
- Page 135 - Protocol 5 animal suffering - is an ulcer by definition a non-healing wound? Why would animals that are only being immunized develop tumours or ulcers?
- Page 139 - Will you be using VDR KO mice up to 14 months? Above you mention "Collectively, the VDR KO animals will not be used more than 5 months of age and with the addition of the custom rescue diet, these mice are not expected to experience adverse effects." "Any new mouse strains exhibiting unexpected harmful phenotypes will be killed..." - what other strains are you planning to use? Need to describe in the GAA section at the beginning of the protocol.
- Page 140 - "No clear improvement in skin lesions greater than 3 mm within 24 hr despite treatment. 20% weight loss from max weight. Body condition <2/5. Signs of incontinence / pilo-erection. Un-responsive to stimuli. Paralysis / loss of limb function. Lumps (internal and external) affecting body function / mobility." - are you expecting any of those signs in any of the lines?
- Page 142 - The sentence beginning "Likewise in the period….." is repeated
- A number of comments were made regarding your Non-Technical Summary which are listed below. Please contact [redacted]) for advice on how to revise the NTS.
    - In general the NTS would benefit from simplification, writing it in more lay language and being more succinct. Please ensure you proof read the final version.
    - It needs to be noted somewhere in the NTS that both sexes of animals are used and adults and juveniles are both used.

- Page 2 - We only need a short description of aim; but you could combine both points into something like "Our aim is to better understand the principles that enable the immune system to detect cancer regardless of origin and produce knowledge that can be translated into effective anti-cancer therapies." You could also consider dropping "regardless of origin"
- Page 2 - for why is it important, you could drop "overall" and start with "Our work". I think it is fine as it's nice and to the point though it technically does not say why the work is important it leaves this implicit assuming the reader will recognise anti-cancer therapies have value.
- Page 3 - Include information on how microbes fit into the project.
- Page 3 - "This 5-year programme for this Laboratory…." could be deleted - the question merely asks for outputs.
- Page 4 - "Dure to this dynamic ecosystem" - typo: dure for due
- Page 4 – include some information on ablation of gut flora.
- Page 4 - "a similar number of genes, many of which are conserved and involved in similar processes" - it's not clear what "conserved" means here
- Page 4 - "Typically, what will be done…" paragraphs: these paragraphs don't read easily as parts of the NTS. A few examples: a) what is the meaning of "characterise" in this context? b) "using imaging methods under normal or treated conditions" - what imaging methods, and what differentiates 'treated' from 'normal' conditions? c) "via appropriate routes (including oral and injection)" - are other routes to be used?
- Page 4 - Most of the paragraph beginning "Typically, in any experiment…" doesn't tell the NTS reader what will be done to the animals, but the final sentence of this paragraph introduces, but doesn't specify, "appropriate surgical procedures." What are these?
- Page 5 – Please use the phrase "humanely killed" rather than killed by schedule 1 method as the public won't know what that is.
- Page 5 - "FELASA guidelines are defined in: "Pain and distress in laboratory rodents and lagomorphs", Report of the Federation of European Laboratory Animal Science Associations working group on pain and distress. " I am not sure what this sentence is trying to say - the question to be answered is expected severities? Could delete this and start with "All the protocols..."
- Page 6 - "Which non-animal alternatives…" paragraph - "bioinformatic analysis" needs explaining for the lay reader of the NTS.
- Page 6 – "How have you estimated the numbers of animals you will use?". This information does not answer the question. Please seek advice from the BSF.
- Page 7 – Consider inclusion of the prior in vitro work.
- Page 7 - perhaps delete "as Postdoctoral Scientist" - not required information.

- Page 7 – Please reword “Based on preliminary data using few mice when no information is available in the literature so that the number of mice utilised in experiments will be reduced to a minimal level.”
- Page 7 - " Based on preliminary data using few mice when no information is available in the literature so that the number of mice utilised in experiments will be reduced to a minimal level" - this does not really make sense though I think the gist is you will pilot using few animals? Could it be clarified?
- Page 7 - Previous work section - this could be simplified by just saying the numbers are based on previous experience and the number of people planned to be working on the project.
- Page 8 - It wasn't clear in the NTS how long mice are housed mice individually (in the ventilated cages) if at all? Can you explain what sort of enrichment you will be providing for them - which I guess would be especially important if they were housed on their own?
- Page 9 - "Other treatments maybe be used…": to what treatments does this sentence refer - treatment for ulcerations and dry & scabbed skin? And are these treatments being applied as advised by NVS/NACWO, or are they being trialled? If the latter, is this appropriate? The public won't understand what NVS or NACWO is. Just use 'vet' and Animal Care and Welfare Officer maybe?
- Page 9 - In the NTS, under "How will you refine the procedures you're using to minimise the welfare costs" add that general or local anaesthesia, sedation or analgesia will be given to mitigate pain and distress. Also antibiotics will be given prior to irradiation and throughout to prevent risk of infection
- Under what will be done to the animals, also add that whole-body or localised radiation will be given at the minimum dose, that animals will sometimes need to be restrained in a jig, have endocropic imaging. I think it might be good to talk about frequency of procedures in the NTS eg: oral gavage either once or twice per day is given for up to 3 months and injections once per day for up to two cycles each of up to max 2 weeks. Also, you need to make it clear some will be housed in germ free colonies, explaining how that affects their health.
- In the NTS, under harms, add that radiation toxicity may occur resulting in reduced mobility, dehydration and inappetence.

Outcome:

- The application cannot proceed in its current format. Revisions are required and the application can only proceed to a another AWERB meeting once the members of the pre-AWERB meeting are satisfied with the revised application.

3. Retrospective Assessments of Project Licences requiring full committee review

3.1. [redacted], The role of inflammation in cerebrovascular disease

Considered: A completed Retrospective Assessment form.

Interviewed: [redacted]

Discussed:
- The licence holder always tries to publish all data from studies.
- Overall, less animals were used in the project than predicted, however more animals were used in some protocols then requested for use.

Outcome: AWERB support submission of the Retrospective Assessment to ASRU.

4. Report on licences processed from 06/10/2022 to 03/11/2022

The following amendments were approved by the executive committee.

4.1. Amendments to Project Licences

[redacted], Immunoregulation During Parasitic Helminth Infection.

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

5.1. The committee were provided with a document showing the status of applications considered previously and those pencilled in for future meetings. Two people pencilled in for the meeting today did not meet deadlines so will be scheduled for later meetings.

5.2. AWERB considered a Project Licence application for [redacted] back in September 2022 where all expect the severe protocol was supported by AWERB. The current Project licence was submitted to the Home Office in October without the severe protocol.

6. Any other business

6.1. Student newspaper

The student newspaper team toured the facility this week. They will be publishing an article.

6.2. Fish in research week

Paul Kasher, working with Understanding Animals Research, will be doing an Instagram take over as part of #fishinresearch week.

6.3. Understanding Animal Research

Mike Addelman is attending the awards on 5 December. We've been shortlisted our work around the EARA 'Be Open Animal research day' campaign (#BOARD22)

6.4. RSPCA lay members forum

A number of members are attending the meeting with the main topics on fulfilling AWERB tasks that relate to Replacement, and self-assessment for AWERBs (particularly relating to the audit process within the Animals in Science Regulation Unit Change Programme). Those AWERB members attending will report back at a future meeting.

The next meeting will be on 15 December 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