CONFIDENTIAL MATERIAL

THE UNIVERSITY OF BIRMINGHAM

Animal Welfare and Ethical Review Body (AWERB)

25th May 2023 (via Zoom)

### MINUTES

### Present:

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| 23/05-01 | Apologies |
| 23/05-02 | MinutesThe minutes of the meeting held on 6th April 2023 were considered by the Committee and were approved. |

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| 23/05-03 | Matters Arising23/04-07/1 *Investigating the inflammatory processes that support formation of atherosclerosis in arterial disease* applicationhas undergone further revisions and will be circulated electronically before submission to ASRU.23/04-07/2 *Investigating the effects of chronic kidney disease on cardiac structure and function* has been submitted to ASRU.*23/04-07-3 Development of Novel Antimicrobial Peptides for Tackling Corneal Infection and Antimicrobial Resistance* has not progressed as there has been no response from the applicant following the committee’s request for further welfare-related details to be added to the paperwork, and AWERB has not approved it for submission. |
| 23/05-04 | Chairperson’s ItemsAWERB are still receiving poorly written applications. The Director of BMSU is replying to applicants, on behalf of the Chair of AWERB, requesting that applicants provide further information. There are nine project applications at various stages of the application process at present. |
| 23/05-05 | Verbal Reports from the Director of BMSU and Named PersonsBMSU has passed a HSE inspection, which focussed on the escape of genetically altered animals, with nothing to report and no recommendations.There are significant issues with the elevator within BMSU, but the Committee should be reassured that BMSU Technicians are working hard to ensure there is no impact on animal welfare. An email has been sent to BMSU users to highlight building works over the coming weeks, and to advise about setting up sensitive experiments or breeding programmes that could be impacted. The new cage washer is due to be installed on 15th June.The roles recognised under ASPA have been reviewed so that there is duplication of staff in each role to ensure cover. This has included adding a new Home Office Liaison Contact to ASPeL, and a new NACWO to the Establishment Licence. There are no health screening issues. The radiation source has changed within BMSU, and the installers have worked with BMSU to ensure the dosage is correct. The equipment was recalibrated on installation, and equivalent doses with the new equipment are known.  |
| 23/05-06 | Report from the Fast Track ProcedureAll applications are uploaded to Teams for comments and are then progressed.For the amendment to “Immune cell migration in health, age and inflammatory arthritis” licence a note has been added explaining the rationale for leaving the animals longer following irradiation and before induction of arthritis. |
| 23/05-07-1 | Project Licence Applications1. *Discovering new ways to treat fungal meningitis*

SummaryThe stated aim of this project is to identify new strategies of activating brain-resistant myeloid cells to help fight fungal meningitis and associated brain inflammation.* Cryptococcal meningitis is the most common cause of fungal brain infection in humans leading to ~100,000 deaths every year.
* The World Health Organisation recently identified the pathogen responsible for this disease (Cryptococcus neoformans) as the top priority fungal pathogen requiring attention for research, health and policy.
* Better treatments for this infection are required, which will rely on a basic understanding of how the immune system mounts a defence to this fungus within the target organ (brain) and how the fungus establishes brain infection.

The Committee raised the following points:The dosage of gamma radiation was discussed and how to ensure that animals aren’t over dosed. It was explained that a routinely used dose will be used initially and pilot studies will be used to determine whether this was a sufficient dosage to achieve the scientific aims. An expert member of the committee will provide follow-up advice to help identify the best starting dosage. Non-infected mice will be used as a control. There was a query whether immunocompromised mice would make a better model for this study. It was acknowledged that no model is perfect and all have advantages and disadvantages, however the proposed approach would provide the baseline data upon which further studies can be developed if required. The number of bone marrow chimeras was discussed, and it was suggested that 1500 is unrealistic for the funding available and project duration.The committee requested clarification on the adverse effects typically seen in this model with there being a concern around signs of meningitis and the immune response that it produces, along with the measurable outputs from the animals. However, it was emphasised that fungal infection burden, along with inflammation and immune cell activity in the brain, can be measured before the animal shows frank signs of meningitis. On the basis that the animals do not reach the late stages of disease, it was agreed that a moderate severity classification is appropriate, but the humane end points need to be clarified in the licence application to better reflect this. The adverse effects associated with Tamoxifen also need to be included.The issue of the role of copper in determining the consequences of fungal infection in this model was raised; it was agreed this may be investigated in the future. It was also queried whether any of this work can be undertaken in vitro rather than in live animals. It was stated that the cell types involved behave very differently in vitro and so unfortunately this is not possible.The type and level of drugs to be administered was discussed, and whether antifungal drugs work alongside immunomodulatory drugs. It was confirmed that they do, as is the case in the clinic where they are prescribed in combination. **Decision: The Committee agreed that further discussions are needed between the scientific expert, NVS, BMSU, NACWO and PI prior to the application being submitted to ASRU. The application will be uploaded to the Teams site at the point of submission.** |
| 23/05-07-2 | 1. *Assessing the impact of autoimmune disease associated fibroblasts on anti-tumour immunity*

SummaryThe stated aim of this project is to understand whether fibroblasts from individuals with an autoimmune disease have the capacity to strengthen the anti-tumour immune response. Fibroblasts are structural cells within tissues that have been shown to suppress the host immune response to cancer but strengthen it in autoimmune disease.* The ecosystem around the tumour cells can often suppress the immune response to cancer – this includes fibroblasts, which are important structural cells that can also modulate the immune system.
* Several cancer-associated fibroblast subtypes have been shown to suppress the anti-tumour immune response, leading to a lack of immune cells in the vicinity of the tumour.
* The aim is to understand whether fibroblasts from an autoimmune disease setting may increase the number of immune cells in the vicinity of tumour cells, and whether this also translates to a subsequent shrinkage of tumours.

The Committee raised the following points:There is an optional step to use the IVIS imaging equipment and it was queried what benefit this gives over simple calliper measurements of tumours. It was explained that whilst the IVIS is used for measuring tumours, it will also allow for the live monitoring of labelled cells through the course of the experiment, rather than harvesting lots of mice at different time points to investigate fibroblasts ex vivo. It was noted that serum from a GA mouse strain is required for the arthritis protocol, but the PPL application does not include breeding of GA mice. The source of this serum was therefore queried and it was explained that it is obtained directly from another UK Establishment under appropriate licence authority.It was queried whether the arthritic animals from which cells are being obtained post-mortem needed to develop arthritis to the full extent. It was explained that the required cells needed to be maximally activated and so the donor animals needed to be killed at the peak of inflammation. Reassurance was provided however that whilst peak arthritis must be reached, the animals will then be humanely killed. The application needs to be altered to explain that a reduced disease burden has been considered and a scientific justification provided as to why this wasn’t possible.It was queried whether there is an expectation for any of the tumours to ulcerate? It was stated that this would be very rare and certainly not a clinical requirement. Nevertheless, it was agreed that ulceration should be added in as a humane endpoint. From a refinement point of view, it was queried whether it would be possible to measure tumours using ultrasound? It was argued that following training, calliper measurements are a standardised method of assessing size and not distressing for the animal. By contrast, there would need a positive reason to use ultrasound because each measurement would require anaesthetic to be given to the animal. It was pointed out that there have been practical issues with assessing fibroblast activity or activation state following cell isolation. As soon as the cells are isolated, they need to grow to get a high enough number to use for experiments, but they lose their phenotype. It was argued that for this reason, cell culture is minimised where possible, and low cell input technologies are used to look at experimental outputs. There was a query regarding whether GA animals should be used, rather than wild type mice acting as both fibroblast donor and host, as this would allow fibroblasts from the donor to be discriminated from those of the host. It was argued that if there are any issues, cells could be tagged to allow differentiation between the two. **Decision: The Committee agreed that further discussions are needed between the NVS, BMSU, NACWO and PI prior to the application being submitted to ASRU. The application will be uploaded to the Teams site at the point of submission.** |
| 23/05-07-3 | 1. *Investigating the regulation of lymphocyte activation and function*

SummaryThe stated aim of this project is to understand how T and B cells sense and respond to their environments in the contexts of health and immunisation.* T and B cells are essential immune cells that function to protect individuals from infections by bacteria and viruses.
* They work together to promote immune responses that last over time (called memory) meaning that after exposure to a germ your immune system is better and more equipped at responding to any potential future infection.
* Through understanding how T and B cells sense and communicate through receptors on their cell surface, we can better understand the fundamentals of immunity.

The Committee raised the following points:More information was requested around the choice of adjuvant. Alum is a standard adjuvant, but there is a need to state which other adjuvants are going to be used. The response was that for this study, alum would be sufficient for most of the protocols, and the cell transfer model may not need to be used. It was explained that any adjuvant to be used needed to be translational to a clinically relevant setting: the pharmaceutical collaborator has a suite of adjuvants, one of which is alum, and they would be able to advise on other clinically relevant ones. Alum has been used at BMSU and no adverse effects have been seen previously using this same route of injection. It was requested that the adverse effects for each individual adjuvant be clarified in the application.The need to inject in the plantar surface of two different feet was questioned as there were concerns that this could result in an animal being forced to walk on a painful foot. Scientific justification for this was provided, but it was also explained that from experience, the injection into the second foot does not cause pain beyond the momentary discomfort of the injection and the animals recover well with no issues with weight bearing on that foot. It was requested that the adverse effects and humane end points be written to reflect this. The region of the foot to be injected was discussed, and it was agreed that the chosen plantar region is the most refined as it does not make contact with the floor. The issue of dose escalation was discussed. This has been developed by another group and the same escalation dosage will be used in the experiments on this Licence. **Decision: The Committee agreed that further discussions are needed between the NVS, BMSU, NACWO and PI prior to the application being submitted to ASRU. The application will be uploaded to the Teams site at the point of submission.** |
| 23/05-08 | Matters relating to the 3RsBMSU 3Rs Focus Group* The Regional Programme Manager has introduced themselves to Birmingham Enterprise and to make them aware of 3Rs technologies with commercial potential.
* BMSU staff have introduced a water bath in the rat playpen, and it has been a success. The rats are demonstrating positive behaviours and we have some impressive images to use at the upcoming 3Rs symposium.
* A member of AWERB has agreed to deliver a zebrafish enrichment talk at the 3Rs Symposium in September.

NC3Rs* Pint of Science took place on 24th May in Birmingham. Two NC3Rs award holders and/or members of their group delivered replacement-focused talks.
* 4 people submitted outline applications for studentships. The Regional Programme Manager will offer 3Rs advice if they are invited to submit full applications.
* NC3Rs event: Not Just Another NAMs Meeting – NAMs from Aspiration to Implementation. Wed 7th Jun 2023, 09:30 – Thu 8th Jun 2023, 13:30. See website for details. <https://nc3rs.org.uk/events/not-just-another-nams-meeting-nams-aspiration-implementation>
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| 23/05-09 | Condition 18 ReportsOne condition 18 report has been submitted since the last AWERB meeting. |
| 23/05-10 | Retrospective ReviewThere were no reviews. |
| 23/05-11 | Any Other Business. A proposal to have meetings in person per year rather than holding all by electronically |
| 23/05-12 | Date of Next MeetingThe date of the next meeting* Thursday 29th June 2023 via zoom
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**GLOSSARY**

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| 3Rs | Replacement, Reduction and Refinement |
| ASRU | Animals in Science Regulation Unit  |
| AWERB | Animal Welfare and Ethical Review Body |
| BMSU | Biomedical Services Unit |
| GA | Genetically Altered |
| GSK | GlaxoSmithKline |
| HSE | Health and Safety Executive |
| NAM | New Approach Methodology |
| NC3Rs | National Centre for the Replacement, Refinement and Reduction of Animals in Research |
| NCTO | Named Competency and Training Officer |
| NACWO | Named Animal Care and Welfare Officer |
| NTS | Non-Technical Summary |
| NVS | Named Veterinary Surgeon |
| PI | Principal Investigator |
| PIL | Personal licence (Procedure Individual Licence) |
| PPLs | Project licence (Procedure Project Licence) |
| UoB | University of Birmingham |