**Handout 5: Session 4 AWERB workshop**

**Introduction**

This is an excerpt from a PPL amendment that has been submitted to the AWERB for review. The project involves breeding and maintenance of GA mice for studies into the mechanisms underlying the development of Multiple Sclerosis. Until now, all experiments have been done ex vivo in cultured cells. The PI now wishes to add a new protocol involving an in vivo CNS injury model.

New text is highlighted in yellow.

The establishment is a medium sized university. Much of the work done is behavioural work using aquatic species (zebrafish). Mouse use is low: there are two research groups (including this one) using brain slices in neurological studies. There are research groups using stress models in mice, and models of muscle disease. Most of this work involves breeding and maintenance of genetically altered mice, behavioural studies and administration of substances. There are no surgical models in use currently.

**Consider the proposed amendment from the point of view of the NVS, and either the NACWO, the NTCO or a PILh.**

**What questions would you ask, and what recommendations would you make?**

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**PPL amendment request: Dr J Smith, AWERB May 2021.**

**Background**

Multiple sclerosis (MS) is a demyelinating disease featuring an autoimmune and inflammatory response that targets oligodendrocytes for destruction, leading to formation of CNS white matter lesions. The remyelination process ultimately fails due to the destructive environment of the lesion and suppression of differentiation of oligodendrocyte progenitor cells (OPCs) into mature myelin-forming cells. Potential MS therapies are aimed at promoting OPC differentiation and/or inhibiting the immune/inflammatory environment.

Novel therapeutic targets are badly needed for MS, for which there is still no cure.

In a previous project, we studied the effects of the protein **Abc1** on myelination in CNS white matter ex vivo. Abc1 demonstrated repair effects in mouse MS models, stimulating new oligodendrocyte generation and concurrent myelination, as well as re-myelination following toxic injury to CNS white matter tissue.

Our data from this previous study confirmed our hypothesis that **Abc1 has a pro-myelinating influence on CNS tissue *in vitro***. These findings are supported by those from other investigators and in different models. Abc1 addition both protected against CNS damage during injury as well as stimulated remyelination after injury (references provided).

We have characterised the expression profile of the three Abc1 receptors, enabling us to determine which receptor type is activated in which cell types. Significantly, we found differing cellular expression profiles of these receptors in CNS glial cells - oligodendrocytes (myelin-forming cells), astrocytes and microglia.

Although we have characterised the receptor expression profile in the mouse brain and in cultured human OPCs, it remains unclear on which cells in the CNS Abc1 acts, and through which receptor, under different conditions, e.g. inflammation or injury. We hypothesise that

* Abc1 acts directly on reception 1 in OPCs to stimulate oligodendrocyte development and myelination,
* Abc1 acts via receptor 2 on astrocytes to suppress their activation,
* Abc1 acts via receptor 3 in macrophages/microglia to stimulate IL-10–driven immune suppression.

All of these hypotheses will be tested using specific single receptor knockout mice.

**Objectives**

We will use WT and GA mice to determine the role of individual receptors in regulating CNS glial cells

**Project plan**

The overall strategy of the project is to use *ex vivo* CNS tissue, both in culture as well as following *in vivo* toxin-induced CNS demyelination, to elucidate the cellular and molecular mechanisms of the myelinating effects of Abc1 receptors and Abc1.

Below, for each Objective of the project, the appropriate **Protocol** involved is stated.

1. **Using Abc1 receptor knockout mice, the roles of the individual receptors in mediating remyelination *in vivo* following demyelinating injury, as well as on glial cell functions in *in vitro* culture.** **Protocol 1:** Single receptor knockout (KO) mice – will be bred and maintained.
   1. Wildtype and KO mice of both sexes at ages P8-12 will be Schedule 1 killed and i*n vitro* organotypic cerebellar slice cultures set up. The role of each receptor in mediating the effects of Abc1 on remyelination will be determined after induction of injury in vitro.
   2. WT and KO mice of both sexes at age 2 months will be Schedule 1 killed and *in vitro* optic nerve cultures set up to determine the role of each receptor on the effect of Abc1 on OPC/oligodendrocyte numbers.
2. **Protocol 2:** *In vivo* focal demyelination model.
   1. Wildtype and KO mice of both sexes at ages 8-10 weeks will be used. Prior to surgery, animals will be randomly assigned to three experimental groups: sham, LPC and LPC + protein M. Sham animals will receive injection of normal saline. Each experimental group will included five time-points (3, 7, 14, 21, and 28 days) post-LPC injection.
   2. All surgeries will be performed under aseptic conditions and with general gas anaesthesia consisting of a mixture of O2 and isoflurane (2%–4%), given through a mask. Anaesthetised mice are placed in a stereotaxic frame using mouse ear bars. Using a Hamilton syringe (26 gauge), 2μl lysolecithin solution (1% LPC in 0.9% NaCl) is stereotaxically injected into the corpus callosum or spinal cord. Each animal is placed in a heated recovery chamber until it recovers, then returned to its cage. Analgesics will be applied post-operatively as standard practice. The procedure is then repeated for the remaining animals.
   3. During the treatment period, animals allocated for histological analysis receive subcutaneous daily injections of 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg) to label proliferating cells. First BrdU injection is performed immediately after the surgery followed by daily injections until the study endpoint.
   4. At the end of each experimental time-point, animals will be Schedule 1 killed and transcardially perfused with 2% paraformaldehyde, and tissues extracted for downstream *ex vivo* analysis.

For Protocol 2 (mouse focal CNS demyelination model, *in vivo*), we plan to use up to 288 mice in total. Here, the experiments will utilise 4 separate mouse lines (1 WT and 3 x GA), each involving 3 treatments, with 6 mice per treatment, and ending the experiment at 4 separate time points. These will be necessary in order to accurately measure the rates of demyelination and demyelination following injury, and therefore crucial for discerning any significant differences between the treatments and between the different mice.

**Refinement**

Our experiments are refined in terms of being mostly *ex vivo* in nature, thus avoiding any experimental procedure on the mice prior to killing. We have already used these mice in the past and the data from them has been peer reviewed and published. Using these unique knockout mice will be fundamental to addressing some of the project’s key questions. Based on many published papers these KO mice are expected to be viable, fertile, normal in size and to not display any gross physical or behavioural abnormalities.

**Protocols**

**Protocol 1 – breeding and maintenance of GA animals (mild severity)** **Protocol**

**What is the maximum number of animals that will be used on this protocol?**

4000(up from 3000)

**Steps**

**Step 1 (mandatory)**

Breeding and maintenance of genetically altered mice by conventional breeding methods.

**Step 2 (optional)**

Tissue biopsy to determine genetic status by one of the following methods: ear punch, blood sampling, hair sampling (AA).

Rarely, due to technical problems during analysis, a second sample may be taken using the least invasive method. Ear biopsy is the default technique.

**Step 3 (optional)**

Maintenance of animals by methods appropriate to their genetic alteration until they reach a maximum of 15 months of age.

**Step 4 (mandatory)**

Animals not used on other protocols will be killed by:

* a Schedule 1 method;
* or by exsanguination under terminal anaesthesia (AC), completed by a Schedule 1 method;
* or by perfusion fixation under terminal anaesthesia (AC).

**Adverse effects**

No adverse effects are expected from these genetic alterations.

**Describe the general humane endpoints that you will apply during the protocol.**

Animals exhibiting unexpected adverse effects will be humanely killed (Schedule 1); if an individual animal exhibits a phenotype of particular scientific interest or concern, advice will be promptly sought from the local HO Inspector.

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**Protocol 2 - In vivo demyelination model (moderate severity)**

**What is the maximum number of animals that will be used on this protocol?**

288

**Protocol steps**

**Step 1 (mandatory)**

Wildtype and KO mice (i.e. 4 phenotypes) of both sexes at ages 8-10 weeks will be used. Prior to surgery, animals will be randomly assigned to two or three experimental groups: (1) sham, (2) LPC and (3) LPC + protein M. Sham animals will receive injection of normal saline. Each experimental group will include four time-points (3, 7, 14 and 21 days) post-injection.

**Step 2 (mandatory )**

All surgeries will be performed under aseptic conditions and under general anaesthesia using isoflurane (2%–4%) (AC); the anaesthetised state will be verified by checking reflexes using a hind-paw pinch. The anaesthetised mice are placed in a stereotaxic frame with mouse ear bars, and incision made. Using a Hamilton syringe (26 gauge), 2μl lysolecithin solution (1% LPC in 0.9% NaCl) is stereotaxically injected into the corpus callosum (coordinates with reference to Bregma point: anterior-posterior (AP): 0.98mm, medial-lateral (ML): 1mm, dorsal-ventral (DV): 2.1mm).

**Step 3 (mandatory)**

Following surgery, each animal is placed in a heated recovery chamber until it recovers, then returned to its cage. The procedure is then repeated for the remaining animals. Analgesic (Lidocaine 1% (AA)) will be applied post-operatively. Additional post-operative care is deemed usually not necessary as the animals are normally fully ambulatory and capable of self-feeding and drinking as soon as they recover from anaesthesia.

**Step 4 (optional)**

During the treatment period, animals allocated for histological analysis receive subcutaneous daily injections of 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg) to label proliferating cells. First BrdU injection is performed immediately after the surgery followed by daily injections until the study endpoint.

**Step 5 (mandatory)**

At the end of each experimental time-point, animals will be Schedule 1 killed or killed under terminal anaesthesia by exsanguination and transcardial perfused with 4% paraformaldehyde (AC); tissues are extracted for downstream *ex vivo* analysis.

**Adverse effects and end points**

Studies show that, after this procedure, animals are normally fully ambulatory and capable of self-feeding and drinking as soon as they recover from anaesthesia. However, animals will be monitored daily for body weight and mobility.

**Describe the general humane endpoints that you will apply during the protocol.**

Any animal showing signs of suffering that are more than minor or transient (as listed in Protocol 1) will be immediately killed by Schedule 1 method.

Animals exhibiting unexpected adverse effects will be killed by a schedule 1 method.