Home Office

PPL Standard Condition 18 Notification Form

Please use this form to notify the Animals in Science Regulation Unit (ASRU) of procedural-related adverse effects that have exceeded or are likely to exceed the severity limitations or controls described in the project licence. ASRU will use this information to determine whether any further action is required.

Do not use to report:

- adverse effects that are due to non-procedural issues;
- potential non-compliance (report by other means);
- issues without adverse welfare consequences.

If you are in doubt whether or not you need to fill in this form, please contact your assigned Inspector.

Please clearly title your document: **'ASRU_establishment name_PPL number_PPLh surname_date_sc18'** and send to your Inspector (e.g. using cjsm) with the subject heading 'PPL SC 18 Notification PPL [Number] – **Check Home Office's Updated Bridging Ways of Working for updated advice on email addresses and subject headings. Cjsm no longer necessary.**

Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Rachel Watson
PPL number	PP6298217
Protocol no	11
Protocol title	Investigation of the effect of test agents delivered directly to the liver
Severity classification	Moderate
1. Date of incident	06/08/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this study is to determine the short- and long-term viability of human iPSC derived hepatocytes (developed by our client) directly injected into NSG mouse livers. It is hoped that these proprietary cells will engraft and support normal liver function, demonstrating their potential as a 'bridging' therapy (test agent) in chronic liver disease patients waiting for transplants. Having been injected into the hepatic portal vein, the viability/pharmacokinetics of the cells in-vivo and their effect on biomarkers will be assessed over at least 21 days by repeated measurement human albumin levels in the mouse serum. 22 female NSG mice have been recruited to this study. As this surgery hasn't been performed by our team before, we performed multiple successful practice sessions on cadavers which were observed by both the NVS and NACWO and organised the presence of the NVS and deputy NACWO during the first live surgeries. We aimed to inject just 4 mice in the first instance, assess animal welfare and recovery rate before continuing with the surgeries using lessons learned. On 06/08/24 the first mouse was given sub-cut Metacam (1.5mg/ml at 5mg/kg) and buprenorphine (0.015mg/ml at 0.1mg/kg) 45 minutes prior to being placed under isoflurane anaesthesia. A laparotomy was performed, and the intestines were gently manipulated out of the peritoneal cavity and onto a sterile wettened gauze pad, exposing the hepatic portal vein. At a dose volume of 10ml/kg, 300ul of hepatocytes suspended in cell media were slowly (~50ul per sec) injected into the vein and haemostatic material used to prevent post-injection bleeding. It was noticed that the mouse had stopped breathing and, after no response to stimulation of the thorax, it was confirmed dead by a sch1 method. We discussed with the NVS and deputy NACWO and decided to operate with a second mouse. As before, the surgery progressed well until the cells were injected, at

	which point the mouse ceased breathing and couldn't be recovered. Both mice had a brief post-mortem. Lungs were checked for signs of congestions and the peritoneal space checked for evidence of portal vein bleeding- no evidence of either was observed. The livers appeared pale and were collected for histopathology.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	After the second mouse died, the NVS provided her opinion on the causes and advised that we pause the surgeries.
5. Cause of Problem, if known	Due to the rapid onset of death in the seconds following the hepatic portal vein injection and the pale appearance of the liver during post-mortem, we (with agreement from the NVS) strongly suspect that an injection volume of 10ml/kg was too high. Also, the cells have a short window of viability and the client requested they were kept cold as much as possible. The temperature, combined with the volume may have contributed to the outcome.
6. Action that has been or will be taken *(For example, formulation of drug changed, supportive treatment given, study design modified). If no action needed, indicate why (for example, none required as study has been terminated and will not be repeated)*	Meetings have been scheduled with the client to discuss how the formulation might be adjusted to reduce the injection volume and increase the solution temperature during delivery.
7. Reported by (name)	Sam Hilton (on behalf of Rachel Watson)
8. Date	07/08/24

**********For official use**********

Inspector’s comments and recommendations:

Comments and recommendations	
Inspector name	
Date	