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	13/08/2024
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. Adverse effects were observed in the first two mice, with both mice dying under anaesthesia during the surgery. Please refer to 'ASRU_University of Cambridge_PP6298217_Watson_070824_SC18' for the first SC18 submitted regarding the first two mice. Having made refinements to the surgical protocol (namely pre-warming the solution and reducing the injection volume) we planned to inject 100 µl of pre-warmed vehicle into one mouse. If well received, the next mouse would receive pre-warmed vehicle + cells. Assuming successful recovery in those animals, the following 3 mice would be subjected to the same protocol. On 13/08/24, the first mouse was given sub-cut Metacam (1.5 mg/ml at 5 mg/kg) and buprenorphine (0.015 mg/ml at 0.1 mg/kg) 30 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. 100 µl of pre-warmed

	vehicle was injected into the hepatic portal vein of the first mouse. Breathing was maintained throughout and, following closure of the surgical wound with absorbable sutures and bupivacaine splash block, the mouse is recovering well (as of 24 hours post-surgery). The same surgical procedure was carried out in the following mouse except that 60 µl pre-warmed vehicle + human iPSC derived hepatocytes were injected. As with the mice the previous week, the mouse ceased breathing almost immediately after the injection was given. Stimulation of the thorax failed to recover her, and death was confirmed by Sch1 method before the liver was collected in formalin for histology. After discussing with the deputy NACWO we decided to try injecting a smaller volume and pre-warm the syringe containing cells for at least 20 minutes prior to being administered. (The previous solution had been pre-warmed for ~3 minutes.) 50 µl of cell mixture was injected, having followed the same surgical procedure as the vehicle injected mouse, and breathing once again ceased post-injection. The mouse was confirmed dead before liver, lungs, heart and brain were collected for histology.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	After the second cell injected mouse died, the decision was taken (with the backing of the NACWO) to stop the surgeries and further investigate the physiological response to the cells before continuing.
5. Cause of Problem, if known	The common denominator for all the mice that died was the inclusion of iPSC derived human hepatocytes in the injected solution. The mouse that was injected with vehicle only survived without issue. As we don't expect any adverse reaction to the biological agent itself in immunocompromised mice, this suggests that the cell density within the solution may be too high.

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 to discuss how the formulation might be adjusted to reduce the cell density in the injected solution.
7. Reported by (name)	Sam Hilton (on behalf of Rachel Watson)
8. Date	14/08/24

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

Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	