



# 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                                                                                                     |
| Establishment Licence number | X81BD37B1                                                                                                                   |
| Name of PPL holder           | Brian Huntly                                                                                                                |
| PPL number                   | PP3042348                                                                                                                   |
| Protocol no                  | 7                                                                                                                           |
| Protocol title               | Study of the impact of cell extrinsic factors on normal, pre-malignant and malignant haematopoiesis through transplantation |
| Severity classification      | Moderate                                                                                                                    |
| 1. Date of incident          | 27-Oct-2023                                                                                                                 |

|                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>2. Species</b>                                                                                               | Mouse                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>3. Brief details of study and how the limits or constraints have been breached</b>                           | <p>the mice were lethally irradiated with 11G splitted into two separate doses on 12<sup>th</sup>-October and were injected with modified murine haematopoetic stem cells via tail vein injection on 13<sup>th</sup>-October. All the mice in the experimental cohort were checked and weighed every other day according to schedule and there was no significant weight loss in any of them. I have attached the weighing sheet screenshot with this report for this animal. On 27<sup>th</sup>-Oct, this mouse was found with abnormal breathing and was cold to touch. The animal was examined by a senior technician and we agreed to cull it by schedule 1. This mouse at the point of death, lost 8.8% of weigh from Max weight and 8.9% from baseline weight taken on 12<sup>th</sup> of October. On 25<sup>th</sup>- October (two days ago) when it was last weighed by us, it did not show any clinical signs nor lost any weight at all. I have also attached the schedule for this study plan which is in compliance with the approved protocol 7 and was signed off by our NACWO at the end of the report. All the steps in the study plan have been done in a timely manner.</p> |
| <b>4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?</b> | <p>From my post-mortem analysis the animal did not succumb to disease so therefore I have not taken advice from NACWO as it doesn't seem necessary. Following this incidence, we are increasing the weighing and monitoring frequency to be daily.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>5. Cause of Problem, if known</b>                                                                            | <p>These mice have been lethally irradiated to allow the transfer of genetically manipulated haematopeotic cells to engraft and if bone marrow transplant failed, the recipient animals would die because of myeloablation. This is likely the cause of this animal falling sick, as I have performed flow cytometry analysis on the culled animals using cells from bone marrow, spleen and blood and I do not find sufficient amount of engrafted cells. Autopsy also showed that the mouse has a very small spleen.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

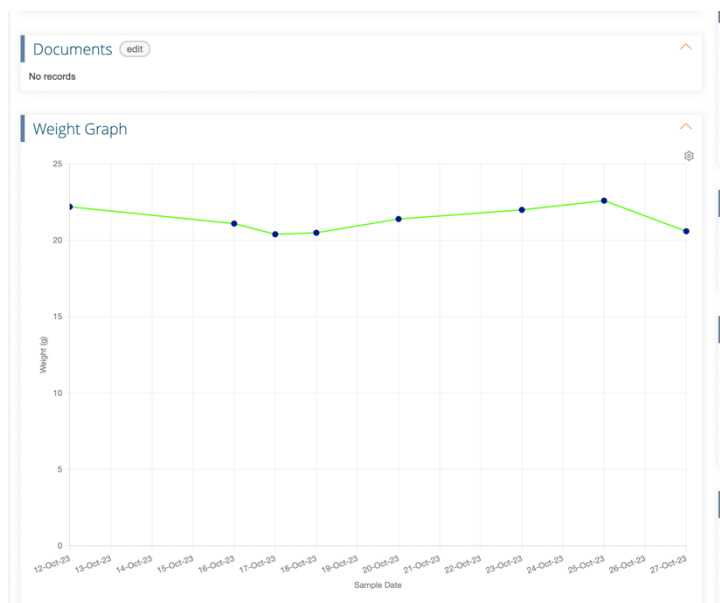
|                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>6. Action that has been or will be taken</b><br>(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) | All the remaining mice are going to be checked daily by us for the first three weeks after irradiation. If the amount of weight loss approaches 20%, or if the animal demonstrates signs of ill-health (piloerection, hunching, reduced activity) that persist for more than 24 hours, the animal will be humanely culled. We will also do so for any of the future studies under the same protocol. |
| <b>7. Reported by (name)</b>                                                                                                                                                                                                                                           | Chun Gong                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>8. Date</b>                                                                                                                                                                                                                                                         | 27-Oct-2023                                                                                                                                                                                                                                                                                                                                                                                          |

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

### Inspector's comments and recommendations:

|                                     |  |
|-------------------------------------|--|
| <b>Comments and recommendations</b> |  |
| <b>Inspector name</b>               |  |
| <b>Date</b>                         |  |
|                                     |  |

Weight chart for the animal involved:



## Schedule for the study plan:

| Row | Complete                                          | Scheduled Date/<br>Day /<br>Schedule Comments                    | Method/Instruction                                                                                                                                                                                       | Procedure/Data Capture                         | Substance /<br>Route /<br>Dose                                    | Cohorts     | Scheduled Task<br>Barcode |
|-----|---------------------------------------------------|------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|-------------------------------------------------------------------|-------------|---------------------------|
| 1   | Vibhuti Agganwal 12-Oct-2023 <a href="#">edit</a> | 11-Oct-2023 (Wed)<br>Day 1                                       | We will weigh the mice pre-irradiation (on the day).                                                                                                                                                     | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 2   | Vibhuti Agganwal 12-Oct-2023 <a href="#">edit</a> | 11-Oct-2023 (Wed)<br>Day 1                                       | Lethal irradiation. (one dose of 5.5Gy, followed by one dose of 5.5Gy). Protocol 7, step 1b.                                                                                                             | Irradiation                                    | 11Gy in total split into 2                                        | All Cohorts |                           |
| 3   | Ryan Asby 13-Oct-2023 <a href="#">edit</a>        | 12-Oct-2023 (Thu)<br>Day 2                                       | Intravenous tail vein injection facilitated by whole body warming. Protocol 7, step 1.                                                                                                                   | Tail vein injection                            | 10 million cells in 200ul PBS                                     | All Cohorts |                           |
| 4   | Vibhuti Agganwal 16-Oct-2023 <a href="#">edit</a> | 16-Oct-2023 (Mon)<br>Day 6<br>Within 7 days post transplant      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 5   | Vibhuti Agganwal 17-Oct-2023 <a href="#">edit</a> | 18-Oct-2023 (Wed)<br>Day 8<br>Within 7-15 days post transplant   | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 6   | Vibhuti Agganwal 18-Oct-2023 <a href="#">edit</a> | 20-Oct-2023 (Fri)<br>Day 10<br>Within 12-20 days post transplant | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 7   | Vibhuti Agganwal 20-Oct-2023 <a href="#">edit</a> | 23-Oct-2023 (Mon)<br>Day 13                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 8   | Vibhuti Agganwal 23-Oct-2023 <a href="#">edit</a> | 25-Oct-2023 (Wed)<br>Day 15                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 9   | Vibhuti Agganwal 25-Oct-2023 <a href="#">edit</a> | 27-Oct-2023 (Fri)<br>Day 17                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 10  | Vibhuti Agganwal 27-Oct-2023 <a href="#">edit</a> | 30-Oct-2023 (Mon)<br>Day 20                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 11  |                                                   | 03-Nov-2023 (Fri)<br>Day 24                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 12  |                                                   | 10-Nov-2023 (Fri)<br>Day 31                                      | A month after Bone marrow transplantation, blood sample will be taken from the mice to track engraftment. Volume to be taken will not exceed 15% of total blood volume within 28 days. protocol 7 step 3 | Blood sampling from superficial vessel         | Saphenous vein less than 16% of total blood volume within 28 days | All Cohorts |                           |
| 13  |                                                   | 10-Nov-2023 (Fri)<br>Day 31                                      | We will check and weigh the mice.                                                                                                                                                                        | Weigh animals                                  |                                                                   | All Cohorts |                           |
| 14  |                                                   | 16-Nov-2023 (Thu)<br>Day 37                                      | Sheep red blood cells injection into the remaining mice in the cohort including Protocol 7, step 2a                                                                                                      | Administration of substances (Intraperitoneal) | 500M sheep red blood cells in 200ul PBS                           | All Cohorts |                           |