PP6298217 SC18 reports summary

PROTOCOL 3 – INVESTIGATION OF THE PHARMACOKINETICS OF TEST AGENTS (NON-INSTRUMENTED)

13 October 2022 (Rat)

- One rat (out of 24 on study) died whilst in a restrainer for a tail vein bleed.
- On postmortem, the rat was found to have an extremely distended GI tract, which could have caused breathing difficulties when restrained.
- Small holes were drilled into the nose cone of the restrainer, with the aim of allowing more air to circulate through the restrainer.

4 October 2023 (Rat)

- Rats were receiving intravenous dosing. The first rat dosed was unconscious upon removal from the restrainer, but was resuscitated. As this was thought to be due to the restraint, a second rat was dosed, during which the tail went limp just before completion of the injection, and the rat was dead upon removal from the restrainer. The first rat was slightly subdued with an unsteady gait 90 minutes following injection, and was therefore killed by a Sch1 method.
- Brain, liver, lung & heart tissue was collected for histological analysis.
- This was using a vehicle found in literature, which had not been used by RxCelerate previously, therefore agreed next steps were to dose a single rat with vehicle only and to monitor for adverse effects.

5 October 2023 (Rat)

- One rat received the vehicle formulation as mentioned in the SC18 report from 4 October 2023. The rat required resuscitation upon removal from the restrainer, and displayed laboured breathing following resuscitation.
- This vehicle formulation was therefore determined to unsuitable for intravenous dosing in rats.

20 March 2025 (Mouse)

- The study was investigating the pharmacokinetics of Leucettinib-21. Following blood sample collection 6 hours post dosing, one mouse (out of three) was observed to display clinical signs of lethargy, hunched posture and a continuous piloerect coat.
- As no clinical signs that are greater than mild or transient are expected on this protocol, the mouse was immediately culled and necropsy performed.
- Histological examination of the liver and lymph nodes indicated an underlying infection unrelated to the dosing.

PROTOCOL 6 – INVESTIGATION OF THE TOLERABILITY OF TEST AGENTS

24 September 2024 (Rat)

- An oral gavage dose was inadvertently administered to the lungs in error. The rat was observed to be limp and frothing at the nose immediately following dosing and was promptly Schedule 1 killed.
- The scientist involved underwent further supervised sessions before being signed off as competent at the technique.

27 September 2024 (Rat)

- An oral gavage dosed was inadvertently administered to the lungs in error. The rat was observed to be unsteady, gasping and frothing at the nose. The rat was removed from the other rats to perform Schedule 1, however its heart stopped beating seconds before the point of cervical dislocation.
- The scientist involved underwent further supervised sessions before being signed off as competent at the technique.

PROTOCOL 8 – INVESTIGATION OF THE BIODISTRIBUTION OF TEST AGENTS

13 May 2025 (mouse)

- Mice were being injected with AAVs via intravenous tail vein injection. Mice were heated for 10 minutes in a heating cabinet set to 38°C prior to being placed into a restrainer for injection. One mouse was unconscious upon removal from the restrainer, with resuscitation unsuccessful and death confirmed by a Schedule 1 method.
- This was not observed in any other animals on study (36 in total).

24 June 2025 (Mouse)

- One mouse reached -11.9% bodyweight compared to peak bodyweight, displaying no clinical signs.
- There were discrepancies in the HEPs within the protocol as to whether the reference bodyweight should be peak or day 0 bodyweight, with the mouse at +2.5% from day 0 bodyweight.
- This SC18 requested to keep alive request permission to keep the mouse on study, which was granted for 14 days.
- The HEPs on the protocol were subsequently clarified.

24 August 2025 (Mouse)

- One mouse was observed to look a little unusual, displaying a mild intermittent hunch and looking to 'vibrate' slightly.
- The mouse remained bright, active and alert and displayed normal behaviour. The mouse had also gained weight since last weighed.
- This SC18 requested to keep alive request permission to keep the mouse on study, which was granted for 14 days.
- The mouse was weighed daily for the remainder of the study, and additional enrichment was provided to the cage as some fighting was observed.

PROTOCOL 10 – INVESTIGATION OF THE EFFECT OF CENTRALLY DELIVERED TEST AGENTS

16 December 2024 (Mouse)

- Following injection into two areas of the hippocampus and recovery from anaesthesia, one mouse displayed lameness in the hind right paw.
- This was not observed in four other mice across two experiments.
- This adverse effect was thought to be due to the area of the brain the needle was passed through and was added to the PPL before any further surgeries were performed.

PROTOCOL 11 – INVESTIGATION OF THE EFFECT OF TEST AGENTS DELIVERED DIRECTLY TO THE LIVER

7 August 2024 (Mouse)

- Two mice were administered human iPSC derived hepatocytes to the liver via the hepatic portal vein.
- This was the first time the procedure had been performed at RxCelerate, with multiple successful practice sessions on cadavers prior to the surgery date.
- The surgeries were performed under NVS supervision.
- Upon injection of the cells to the first mouse, it was noted that the mouse had stopped breathing. After no response to stimulation of the thorax, death was confirmed by a Schedule 1 method.
- After discussion with the NVS, surgery was performed on the second mouse, with a similar outcome. It was hypothesised that the injection volume (10 ml/kg) may be too high.

14 August 2024 (Mouse)

- Following on from the surgeries covered by the SC18 dated 7 August 2024, a mouse was successfully injected with 100 µl vehicle solution.
- However, upon injection of a mouse with 60 µl vehicle + iPSCs, the mouse ceased breathing almost immediately after the injection was administered.

- After discussion with the deputy NACWO, a further surgery was performed, injecting 30 µl vehicle + cells after 20 minutes prewarming of the solution. This mouse also stopped breathing post injection.
- Tissues were collected for histological analysis and surgeries were paused pending histological results.
- It was hypothesised that the cell density within the dosing solution may be too high, with the histological analysis supporting this hypothesis.

22 October 2024 (Mouse)

- Following on from the surgeries covered by the SC18s dated 7 & 14 August 2024, a cohort of 6 mice had been successfully administered cells via the intraportal vein.
- Upon surgical administration of cells to another cohort of animals, one mouse ceased breathing ~1 minutes following injection of cells. Resuscitation was unsuccessful, and death was confirmed by a Schedule 1 method.
- Another mouse recovered well initially post surgery, being active when observed 1 hour post surgery. However, she was found unresponsive 1.5 hours post surgery and was immediately killed by a Schedule 1 method.
- Despite some success, it was hypothesised that the cell density may still be the source of the issue and this was reduced further for future surgeries.