Retrospective Review of Project Licences

The information you provide in or attached to this document must reflect data generated only from work carried out under the licence under review. A copy of the final agreed version of this document and any advice provided by the University AWERB in relation to this document will be placed on file. Retrospective Reviews may be requested under the Freedom of Information Act. If this is the case, we will contact you.

1. General Information:

Project licence holder to complete if information provided is incorrect

Name:	Dr Rachel Watson	
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Project number	PP6298217	
Project title	Profiling of test agents in rodents in a drug discovery platform	
Project purpose	The aim of this project is to generate data on the pharmacokinetics, pharmacodynamics and tolerability of test agents for the disease areas listed in this licence, and the effect of test agents in rodents over a longer period of dosing.	

Date licence was granted	3rd February 2022
Date Retrospective Review was sent to the PPLh	20th August 2025

Please tick one of the options below:

A		My licence is due to expire in 12 to 15 months' time and I have no plans to write a new licence
B	✓	My licence is due to expire in 12 to 15 months' time and I plan to write a new project licence to continue my research
C		I plan to request the revocation of my licence before its natural expiry date
D		I will no longer be the project licence holder but, another person will continue to hold the licence. The name of the new licence holder will be:
E		The AWERB Committee has asked for this Retrospective Review.

2. Animal Welfare/Recorded Harms:

2.1 Using the table below please provide the information requested.
How to complete this table: Return of procedures count animal numbers from 1st January to 31st December. In the table below if your licence was granted part way through the year count year one as the time from

when the licence was granted to the 31st December of that year. Year two will be from January to December etc. until the final year for which the report is requested and count from 1st January to the requested report date. Please insert rows for additional protocols as necessary. For any animals that experienced a higher level of severity than that of the protocol please highlight these figures in **bold.**

Protocol No.	Species	Protocol severity category	Total number	Actual number used	Actual severity recorded					
					Non-recovery	Sub Threshold	Mild	Moderate	Severe	Totals
1	Mice	Mild	500	1st year	59		166			225
				2nd year	23		8			31
				3rd year	13					13
				4th year	48		2			50
				Total	143		176			319
1	Rats	Mild	200	1st year	7					7
				2nd year	1					1
				3rd year	10					10
				4th year	10					10
				Total	28					28
2	Mice	Mild	250	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
2	Rats	Mild	100	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
3	Mice	Mild	750	1st year			51			51
				2nd year			18			18
				3rd year			126	1		127
				4th year			21			21
				Total			216	1		217
3	Rats	Mild	500	1st year			72			72
				2nd year			18	5	1	24
				3rd year			16			16
				4th year						
				Total			106	5	1	112
4	Mice	Moderate	100	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
4	Rats	Moderate	250	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
5	Mice	Mild	150	1st year						
				2nd year						

				3rd year						
				4th year						
				Total						
5	Rats	Mild	150	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
6	Mice	Moderate	700	1st year			104	2		106
				2nd year						
				3rd year			25			25
				4th year			166			166
				Total			295	2		297
6	Rats	Moderate	300	1st year			23			23
				2nd year						
				3rd year			35	6	1	42
				4th year						
				Total			58	6	1	65
7	Mice	Moderate	1000	1st year			160			160
				2nd year			136			136
				3rd year			119	1		120
				4th year			29			29
				Total			444	1		445
7	Rats	Moderate	500	1st year						
				2nd year			140			140
				3rd year			72			72
				4th year			22			22
				Total			234			234
8	Mice	Mild	1000	1st year						
				2nd year			43			43
				3rd year			27			27
				4th year			110	2		112
				Total			180	2		182
9	Mice	Moderate	200	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
9	Rats	Moderate	200	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
10	Mice	Moderate	300	1st year						
				2nd year						
				3rd year			1	5		6
				4th year						
				Total			1	5		6
10	Rats	Moderate	200	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						

11	Mice	Moderate	500	1st year						
				2nd year			8	13	1	22
				3rd year						
				4th year						
				Total			8	13	1	22
12	Mice	Mild	500	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
13	Mice	Moderate	300	1st year						
				2nd year						
				3rd year						
				4th year			12			12
				Total			12			12
14	Mice	Moderate	300	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
									Whole Total	1939

*If actual severity data has not been formally assigned at the point the Retrospective Review is undertaken, the columns can be used to indicate the estimated severity for each of the categories.

2.2 Does the information provided in the table reflect the projected incidence of harm provided in the adverse effects for each protocol? If not, please indicate where this was not the case and whether the projected level of suffering was more or less than you anticipated when you first wrote the licence. If the severity category for a protocol was amended during the tenure of the licence, please include this information in the reasons box.

Protocol number	Severity higher/ same/ lower than anticipated	Reason
1	Severity as anticipated	55% mice returned as mild due to continued use from another PPL, whilst lifetime experience was mild, the experience whilst under this protocol was non-recovery. The severity of this protocol was also altered to mild in 2025 to allow the addition of a step for aged animals. This is separate to the above animals, who were returned as mild due to their experience on the PPL on which they were bred.
2		Protocol not used
3	Severity as anticipated	5 mice and 2 rats covered by SC18 where severity limits were exceeded
4		Protocol not used
5		Protocol not used
6	Severity lower than anticipated	It was anticipated that 75% animals would experience moderate severity, but this has been <1% for mice and 10% for rats . This is due to a large proportion of the drugs being tested being well tolerated even at higher doses. This greater tolerability could be due to a greater proportion of newer types of test agents which are less likely to cause adverse effects being tested (e.g. antibodies), rather than traditional small molecule drugs.

7	Severity lower than anticipated	It was anticipated that 20% animals would experience moderate severity, however this has been <1%. This is due to the cancellation of studies involving implantation of slow-release devices or hydrodynamic injections for delivery of test agents.
8	Severity as anticipated	Two mice returned as moderate, covered by SC18.
9		Protocol not used
10	Severity as anticipated	Severity as expected. 1 mouse was recorded as mild due to death under anaesthesia – the lifetime experience for this mouse was therefore mild as the adverse effect occurred whilst the animal was unconscious, but would have been the anticipated severity of moderate had the mouse recovered from anaesthesia.
11	Severity as anticipated	Severity as expected. 8 mice were recorded as mild due to death under anaesthesia – the lifetime experience for these mice was therefore mild as the adverse effect occurred whilst the animal was unconscious, but would have been the anticipated severity of moderate had the mouse recovered from anaesthesia.
12		Protocol not used
13	Severity lower than anticipated	The adverse effects observed from donors tested so far have been lower than anticipated based on literature.
14		Protocol not used

Describe any unexpected adverse effects and include the percentage and the number of animals affected. How were these episodes managed and recorded? If any of these resulted in a Project Licence Standard Condition 18 report, please indicate which were reported under Condition 18 and the date (also see Section 5.2).

2.3

A full summary list, along with copies of each SC18 are provided with this review.

Protocol 3:

- One rat (1/24 on study, 4%) died whilst in a restrainer for a tail vein bleed. On post-mortem, 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 to assist with air circulation.
  SC18: 13/10/2022
- Three rats died or were Sch1 killed due to adverse reaction to the vehicle of a dosing solution. The first two rats had been dosed with test agent formulated in the vehicle dosing solution. After observing the adverse reactions (loss of consciousness in restrainer, unsteady gait, laboured breathing), the third rat was dosed with vehicle only. Following a adverse reaction in the vehicle only treated animal, this vehicle was not administered to any further animals.
  2x SC18: 04/10/2023 & 05/10/2023
- One mouse (1/3 in that group, 1/6 on study total, 17%) died due to clinical signs of lethargy, hunched posture and piloerection ~6 hours post dosing with test agent. Histological examination of the liver & lymph nodes indicated an underlying infection unrelated to the dosing, therefore no further action was required.
  SC18: 20/03/2025
- Overall, <2% animals under this protocol experienced adverse effects.

Protocol 6:

- Two rats were Sch1 killed due to oral gavage doses being inadvertently administered to the lungs in error (2/42 on study, 5%). Both staff members involved underwent further supervised training session and reassessment for the technique.
  2x SC18: 24/09/2024 & 27/09/2024
- Overall, <1% of total animals under this protocol experienced adverse effects.

Protocol 8:

- One mouse (1/38, 3%) was unconscious upon removal from the restrainer following intravenous injection, with resuscitation unsuccessful and death confirmed by a Schedule 1 method. This reaction was not observed in any other mice in the study. Two competent staff members were in attendance, who were able to confirm that technical error was not the cause of the death.
  SC18: 13/05/2025
- One mouse (1/24, 4%) was observed to look unusual, displaying a mild intermittent hunch and looking to ‘vibrate’. The mouse was bright alert and active and continued to gain weight. A SC18 was submitted to request to keep the mouse alive, which was granted. The mouse was weighed and monitored daily for the remainder of the study.
  SC18: 24/08/2025
  Overall, <2% animals under this protocol experienced adverse effects.

Protocol 10:

- Following injection into two areas of the hippocampus and recovery from anaesthesia, a mouse displayed lameness in the hind right paw (1/2 in this study, 50%; 17% of total number of animals on protocol). An amendment was made to to include this potential adverse effect on the PPL, as there were no possible alterations to the procedure possible to mitigate against this outcome.
  SC18: 16/12/2024

Protocol 11:

- Six mice died or were Schedule 1 killed due to adverse effects observed when optimising the delivery of a cell-based test agent by intraportal injection (6/22, 27%). It was initially hypothesised that the injection volume was too high, and subsequently, a smaller volume was confirmed to be well tolerated using vehicle-only injections. Upon further adverse effects, it was

hypothesised that the cell density within the dosing solution was too high, potentially causing a blockage and adverse effects due to obstructed circulation. The cell density was lowered and mice were treated in a staggered fashion to determine a tolerable cell density Histological analysis of the liver and other organs was also performed. 3x SC18: 07/08/2024, 14/08/2024, 22/10/2024

2.4 What percentage and number of animals had to be killed or died unexpectedly before the end of the experiment? Please provide a brief summary of each incident by protocol(s) where this occurred.

Protocol No.	% and number of animals killed or unexpectedly found dead
3	1 rat killed whilst in restrainer for tail vein bleed (4% study, <1% rats on protocol, <1% total animals on protocol).
3	3 rats died due to adverse reaction to vehicle dosing solution (2% rats on protocol, <1% total animals on protocol).
3	1 mouse died due to clinical signs 6 hours post-dosing, post-mortem indicated an underlying infection unrelated to the dosing (<1% mice on protocol, <1% total animals on protocol).
6	2 rats died due to oral gavage doses being inadvertently administered to the lungs in error (5% rats on study, 3% rats on protocol, <1% total animals on protocol).
8	1 mouse unconscious upon removal from restrainer following i.v. injection (3% mice on study, 1% mice on protocol (mouse only protocol)
10	1 mouse displayed lameness in the right hind paw following direct brain injection and was sch1 killed (50% mouse cohort, 17% mice on protocol)
11	6 mice died or were Sch1 killed due to adverse effects observed when optimising the delivery of a cell-based test agent by intraportal injection (27% mice on protocol)

2.5 Were your estimated animal numbers accurately reflected in your actual animal usage as provided in Table 2.1 above? ~~YES~~ **NO**

If no, please explain the why there was a difference. Please do this on a protocol-by-protocol basis.

Protocol No.	If your numbers were less that estimated please explain why.
2	This protocol has not been used due to halting of the main programme of work utilising this protocol. However, a new programme may require use of this protocol in 2026.
4	This protocol has not been used to date due to cancellation of the programmes using cannulated animals. However, this method has been recommended to a client recently and is expected to be required in 2026.
5	This protocol has not been used as all studies under this objective have been tolerability studies performed under protocol 6. This protocol will not be included in the new PPL.
9	This protocol has not been used to date, due to studies for which the protocol for added to the PPL being cancelled after preliminary work demonstrated that the test agents were not suitable for further development as a therapeutic. However, we are in discussions with a client regarding running work under this protocol, which is expected to be performed during 2026.
11	Lower than anticipated numbers have been used on this protocol due to the results meaning that the test agent being used needed to be improved before further work is performed.
12	This protocol has not yet been used due to cancellation of the work programme by the client for whom the protocol was designed. However, the protocol is expected to be used going forward as early discussions with several new clients have indicated that work under this protocol will be required.
13	Lower than anticipated number have been used on this protocol due to confirmation of a suitable human donor for engraftment studies very rapidly, and studies for an existing client coming to an end sooner than anticipated.

14	This protocol has not been used due to cancellation of the work programme by the client for whom the protocol was designed. However, other clients have expressed an interest in work of this kind that would fit within the PPL objectives, therefore this protocol may be used prior to licence expiry.

2.6 Have you re-used animals? ~~YES~~ **NO**

If so, why have you re-used animals and what has been the impact of re-use on the welfare of your animals? Please also provide the species and number of animals re-used by protocol where re-use occurred.

Species	Protocol No. on which animals where/will be returned as re-used, why and what impact, if any, this re-use had on the welfare of your animals

2.7 Has there been any animal wastage? ~~YES~~ **NO**

For example, this may occur when breeding and maintaining genetically altered animals or when animals have been ordered and cannot be used for some technical reason.

Where wastage has occurred, please explain the circumstances and nature of the wastage plus an indication of the numbers of animals. Please also provide either the number or percentage of animals that were wasted.

Circumstances and nature: All animals were bought in or moved from a breeding PPL for specific studies, therefore there was no animal wastage on this PPL.
Estimated number or percentage of animals wasted and if appropriate provide this information by protocol: 0

2.8 For licences that authorise the breeding and maintenance of genetically altered animals:

a) explain how you optimise breeding efficiency, and
b) explain what use is made of any excess stock animals, and
c) explain if, and how, you have used any transgene repository – national, international, or internal.

N/A

2.9 If you breed genetically altered animals are your strains recorded on a database (e.g., MCMS) that can be

a) accessed by other researchers at the University? **YES** **NO**
b) accessed by researchers outside the University? **YES** **NO**
c) would you allow other researchers at the University to use your strains? **YES** **NO**
d) would you allow external researchers to use your strains with an MTA applied? **YES** **NO**

If you have answered YES to any of these questions, please elaborate below:

N/A

If you have answered NO to any of these questions, please explain why.

N/A

2.10 Have you been able to refine and/or reduce the impact of contingent harms arising from your use of animals (contingent harms are harms for example that arise from the transportation of animals, single housing animals or housing animals in non-Code of Practice accommodation, etc.). ~~YES~~ **NO**

Please explain/provide examples.

Transportation of animals by suppliers according to standard conditions.
Single housing is avoided wherever possible.
Animals always housed in Code of Practice accommodation.

2.11 By species and percentage please indicate the source of your animals.

Species	Source	Approx. percentage
Mouse	Charles River (UK)	84.4%
	Internal PP0760009	15.5%
	St Louis University	0.1%
Rat	Charles River	100%

2.12 During the tenure of the licence have you requested an amendment to increase/decrease animal numbers? **YES** ~~NO~~

If so, which protocols were amended and were numbers increased or decreased, and by how much?

Protocol No.	Original estimated Number	Amended estimated number
1	250	500
7	250	500

If you have increased or decreased animal numbers, please explain why this was necessary?

It was necessary to increase the animal numbers on protocol 1 to provide sufficient samples for optimisation of our *ex vivo* assay for screening our PiZ (alpha 1 anti-trypsin deficient) mice. The mice are screened via blood sample at 3-4 weeks to check levels of a biomarker determining for which studies they are suitable for, and the same assay is also used to determine the effect of test agents on biomarkers (under protocol 7 of this PPL). When we refreshed the colony by bringing in new mice, their previously recorded biomarker levels did not match values from our in-house assay. Therefore, blood samples from the refreshed colony were collected under this protocol to optimise the assay and ensure it was suitable for both screening of colony animals and investigating the effect of test agents on the biomarker.

It was necessary to increase the animal numbers on protocol 7 to run a large series of studies for a well-established client who identified some side effects that may compromise the planned progression of their test agent into clinical trials. It was important to investigate the changes in biomarkers after test agent administration, with large blood samples required for the analyses meaning that large numbers of rats were required.

2.13 During the tenure of this licence have you requested an amendment to increase/decrease the severity of any protocols? **YES** ~~NO~~

If so, which protocols were amended and were the severity categories increased or decreased?

Protocol No.	Original severity category	Amended severity category
1	Non-recovery	Mild

If you have increased or decreased the severity category of any of your protocols why was this necessary?

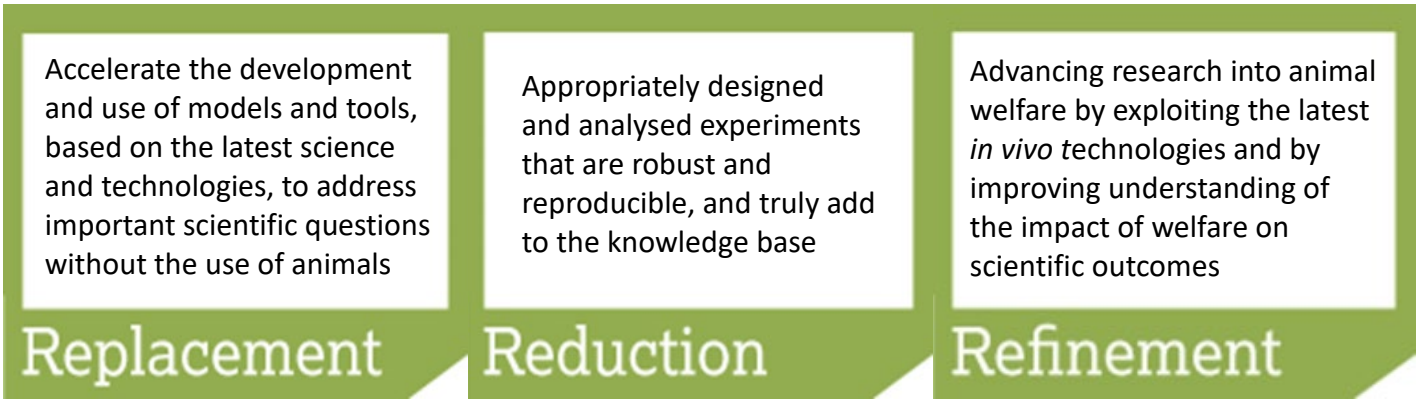
The change of severity was needed to allow use of aged animals under protocol 1. Aged animals have an increased range of potential in-life harms related to ageing e.g. dermatitis but were required for collection of terminal samples capturing ageing-related phenotypes, therefore a new step was added to protocol 1 to allow the housing of aged animals prior to the non-recovery procedure.

3. **3Rs:**

In this section, please explain what steps you have taken during the tenure of your licence that resulted in 3Rs gains. This might include working with collaborators to refine your model, changing the anaesthetic regime or equipment, etc. Consider every change you have made and its impact on the 3Rs – even small changes and initiatives can result in 3Rs gains.

The following definitions come from the NC3Rs website and are included to help you provide information under the most appropriate R below:

Definitions



3.1 Have you attempted or been able to replace any of your techniques or models with non-animal alternatives? ~~YES~~ **NO**

Please explain.

The work undertaken in this project cannot be fully replaced with *in vitro* or *ex vivo* models. Wherever possible, work is performed in *in vitro* or *ex vivo* models prior to animal work to ensure that the *in vivo* studies are well designed and that the maximum amount of data is obtained from each study.

Use of animals is essential to measure how much drug gets into the body and how the body acts on it (pharmacokinetics). Use of animals is essential to measure how much drug gets into the body and how the body acts on it (pharmacokinetics). Understanding this is an essential part of the drug discovery process to help understand and predict drug action. The same is true for tolerability, biomarker, target engagement and biodistribution studies. Whilst thorough testing is performed prior to animal studies, and modelling both via computational and non-animal laboratory methods is continuously improving, it is not possible to fully predict how the action of a test agent will be different within the complex system of a living animal compared to simpler models.

3.2 Have you been able to reduce your animal usage? **YES** ~~NO~~

If so, how have you achieved this (e.g., through experimental design, changes to the regulated procedures used)? Please give an indication of the actual numbers reduced.

Work prior to the animal studies can include target validation and/or testing the effect on a suitable cell line, to check that the potential drug is acting as expected. This work ensures that test agents that are unlikely to be viable for use as drugs in humans are ruled out before moving into animal studies, therefore reducing the total number of animal studies and the total number of animals used. It is hard to accurately estimate the reduction in number of animals due to the *in vitro* testing, but there could have been 2-5x the number of studies required if the *in vitro* testing was less extensive and therefore that fewer test agents were ruled out prior to animal testing.

The running of smaller tolerability studies also ensures that the dosage is set at an appropriate level for larger scale studies looking at the effect on biomarkers, to ensure no animals are wasted in these larger scale studies.

We have optimised the downstream analysis of PK study samples to allow smaller sample sizes to be collected. This is particularly relevant for mice, where previously multiple mice had to be dosed with the same drug to be able to collect samples at enough different times after dosing to perform the full PK analysis. Being able to use smaller samples means that the total number of samples that can be collected from each mouse is higher, therefore fewer mice are required in total.

We also take as many tissue samples from animals at the end of studies which can be used for pilot read outs. This can mean that for example tolerability studies can be used as a pilot for studies looking at the effect on biomarkers, rather than needing to run a separate pilot study. It also means that the number of mice required under protocol 1 for the collection of tissues for *ex vivo* analysis is reduced.

3.3 Are you involved with any tissue sharing either within the University or more widely?

Please explain:

Where possible, we have made animals available locally for tissue collection or post-mortem training, although often we are collecting such a large collection of tissues from each animal that sharing is not possible.

We have recently made use of aged breeding animals under a non-reg form to perform pilot *ex vivo* work ahead of a study under protocol 8. This allowed laboratory methods to be optimised ahead of the study without the use of any additional mice.

We are part of the 3Rs mailing list and make use of this where possible.

3.4 Please LIST the techniques and/or animal models you have used on your licence (include GA models). Later on, you will be asked about the 3Rs achievements – please do not detail your 3Rs achievements in this box.

- Dosing by the oral route by gavage or in the drinking water
- Dosing by intraperitoneal injection
- Dosing by subcutaneous injection
- Dosing by intravenous injection
- Dosing by intramuscular injection
- Administration of immune cells by intravenous injection
- Administration to the liver via portal vein injection
- Administration to the brain by direct injection
- Sampling of blood from a superficial vessel (tail vein)

- Non-invasive imaging (IVIS)
- Ageing
- Withdrawal of a terminal blood sample by cardiac puncture
- Withdrawal of a terminal blood sample via the IVC
- Schedule 1 methods of killing
- Exsanguination under terminal anaesthesia followed by confirmation of death

Mice:
WT: C57Bl/6J
GA: NSG, PiZ (alpha-1 antitrypsin deficient), APOL-G1 expressing mice

Rat:
WT: Sprague Dawley, Wistar (Han)

3.5 How successful have these techniques and/or models been?

The techniques and studies run under this licence have been very successful. We have tested over 90 test agents through the various protocols on the licence and have already fulfilled the target number of compounds to be tested for each objective except one, with over a year still to run on the PPL.

To provide a few highlights of particular successes:

The objective with the most activity on the licence has been the investigation of the effect of test agents on biomarkers, where over 30 test agents have been tested, with the objective for the licence being to test at least 10. We have designed experiments to minimise animal usage and have been able to investigate this high number of test agents with only one amendment to increase the number of rats on the protocol.

1. The study for which the amendment to increase numbers was required was a particularly key study in the development of a novel test agent. This test agent was planned to enter clinical trials in the near future, however, the work run under the biomarkers programme identified a serious side effect that meant that the test agent was not suitable to progress to clinical trials, which was vital to discover ahead of the test agent being dosed in humans.
2. This work has followed on to test alternative treatments targeting the same pathways, with work under the licence allowing the identification of test agents which have the desired effect on biomarkers without the undesired side effects on other biomarkers, therefore progressing work on finding a new treatment for a disease with a large unmet clinical need.

The addition of the protocol to identify suitable donors for establishment of humanised models for the profiling of test agents has allowed the profiling of several donors and the identification of both suitable and unsuitable donors. This has allowed test agents to be profiled in humanised models, which has provided valuable information on the likely success of test agents which target the human immune system. This extra information gained by the use of humanised mice demonstrated that the action in the intact circulatory system did not mimic that in the in vitro laboratory situation as closely as expected, which had a great influence on the next steps for the development of the test agent.

Work performed under the PK, tolerability and biomarker objectives has been incorporated into regulatory submissions for the progression of test agents to the next stages of development.

3.6 What limitations, if any, have you encountered? Were you able to overcome these limitations and, if so, how?

NSG hock issues: As has been observed across UBS, NSG mice experience hock issues, which can become limiting to the experiment depending on their severity. We worked alongside the Mira team to monitor and refine handling of NSG mice, including housing on alpha-dri bedding, introduction of sponges for scruffing, provision of additional enrichment (nesting material changed more frequently, chew blocks provided) and application of sudocrem daily to hock lesions. These refinements allowed a greater proportion of NSG mice to complete studies.

Sudden death of mice during direct liver injection surgery: When initially optimising the direct liver injection protocol, we found that mice were not surviving the procedure. We reduced both the volume injected and the cell density to overcome this issue.

Vehicle adverse effects: Rats experienced adverse reactions to a particular vehicle used in a PK study. According to the literature, this vehicle has been successfully used in vivo; however, we were able to confirm that in our hands, the vehicle, and not the test agents the vehicle was being used to carry, caused the adverse effects. In collaboration with our in-house in vitro and chemistry teams, we performed further work to optimise alternative formulations which enabled in vivo evaluation of two of the three test agents in the study..

3.7 Have you attempted or been able to refine any of your techniques or models?

YES ~~NO~~

Please provide a list of techniques or models you have been able to refine and the refinements you have made. Please indicate if you have published or disseminated this information during the tenure of this licence and attach a copy of the publication.

We include refinement of models as an inherent part of the process when performing any animal work at RxCelerate.

We worked together with Mira staff on the refinement of handling of NSG mice to help alleviate hock issues. This included using alpha-dri bedding, using sponges for scruffing of the animals and applying sudocrem to any hocks showing problems, as well as using a scoring system to monitor progression of any hock issues. We presented a poster on the hock issues in NSG mice at the LASA conference in 2024 and are currently working on a publication to disseminate information around this issue.

When performing tolerability studies using test agents which cause weight loss, we have incorporated Complan into our dietary supplementation options to support maintenance of body weight and condition. Complan is a high calorie nutrition drink which is highly palatable to mice.

3.8 Do you use systems to monitor the welfare of your experimental animals (e.g., scoring sheet)?

YES ~~NO~~

If not, why?

N/A

If you do use a welfare monitoring system, what do you use? Did you refine or modify your system during the tenure of your licence and what impact did this have?

We use in-house daily record sheets for monitoring body weight of animals on a study. These sheets have inbuilt calculations for the percentage change in body weight from the appropriate reference point and highlights mice which may require dietary supplementation or where the humane end point is being approached or reached. These record sheets are also used for the recording of any adverse effects and/or clinical signs.

For work involving the injection of human immune cells to mice, we have developed a GvHD (graft versus host disease) scoring sheet to monitor for any disease developed due to a reaction of recipient mice to the injected cells. We refined the scoring sheet over the first few studies as we sought to reduce ambiguity and subjectivity in the scoring criteria.

We also implemented the hock scoring system developed within UBS. For studies including administration of human immune cells in NSG mice, we created a combined template to allow recording of both GvHD symptoms and hock issues in one MCMS form.

3.9 Were the end points detailed in your project licence appropriate? **YES** ~~NO~~
Have you needed to modify them during the tenure of your licence? **YES** ~~NO~~

Please explain.

An amendment was made to add the standard wording for the adverse effects associated with NSG mice hock issues.

An amendment was made to add new adverse effects related to limb paralysis following brain injection after observations (and SC18) during pilot work.

3.10 Please review the non-schedule 1 methods of killing authorised on the protocols on your licence. In the box below, list each method and beside each clearly indicate which you have used? Examples of non-schedule 1 methods include: exsanguination, decapitation and perfusion fixation.

Exsanguination under terminal anaesthesia followed by confirmation of death by one of the confirmatory methods from Schedule 1 has been used for animals following withdrawal of a terminal blood sample from the IVC.

Perfusion fixation under terminal anaesthesia is also authorised on the licence but has not been used.

Were these killing methods the most refined? **YES** ~~NO~~
Were they the most appropriate for your experimental data? **YES** ~~NO~~

If you have answered NO to either of the above questions, please explain. If you were able to refine the way your animals were killed, please explain how this was achieved.

N/A

3.11 Have you received awards or funding specifically targeted at the 3Rs? ~~YES~~ **NO**

If so what was the source of your funding, when did you receive it and what did the funding allow you to achieve?

N/A

The AWERB 3Rs committee is particularly interested in this section of your review because information you provided here could be of interest and use to other researchers at the University.

Information that you have included in this section will be included in the UBS 3Rs Search Tool unless you complete the box below. UBS will only add information to the UBS 3Rs Search Tool section on the UBS website that University staff can log into using their Raven Access. You should however disseminate all 3Rs finding more widely.

I do not agree to information provided in this section to be added to the UBS 3Rs Search Tool for the following reason(s).

4. Scientific Progress and Benefit Achievements:

4.1 Taking into consideration that your licence may not be due to expire, has your research progressed as you expected? **YES** ~~NO~~

4.2 When your licence is due to expire will you have completed the programme of work described in this licence? **YES** ~~NO~~

4.3 Regardless of whether you managed to complete your programme of work, what have you achieved/discovered during the time since this licence was granted? (Please copy your key scientific questions/objectives which appear at the beginning of Part D, or in the Action Plan, of your project licence into the box below and against each explain what you have achieved/discovered).

The numbers of test agents given below is accurate as of December 2025 with 14 months still left on the PPL.

Objective 1: To produce biological samples for ex vivo analysis
This objective has been achieved, with biological samples regularly generated for ex vivo analysis. These samples have facilitated the validation of test agents before moving into animal models and allowed optimisation and validation of ex vivo assays relevant for other studies.

Objective 2: To determine the pharmacokinetic parameters of at least 20 test agents
We have determined the pharmacokinetic profiles of 25 compounds. Several of these test agents have then progressed into further studies, both on this licence and on other licences covering work in disease models.

Objective 3: To determine the safety and tolerability of at least 10 test agents
We have determined the tolerability of 13 test agents. The safety protocol (protocol 5) has not been used and will not be included on the new PPL. Several test agents progressed to further studies under Objective 4 and on other licences covering work in disease models.

Objective 4: To investigate the effect of at least 10 test agents on biomarkers
We have investigated the effect of 29 test agents on biomarkers. This work has generated promising results for several new therapeutics, enabling their progression to testing in relevant disease models. Conversely, several tested agents have proven unsuitable for progression to clinical trials or further animal work in disease models.

Objective 5: To determine the biodistribution of at least 5 test agents
We have determined the biodistribution of 9 test agents, with further plans to test several more imminently. Analysis of biodistribution helped in determining which of the test agents are likely to be most efficacious at treating the target disease.

Objective 6: To determine the target engagement of at least 2 test agents

We have determined the target engagement of 1 test agent so far. This provided valuable information that target engagement in the animal with an intact circulatory system was lower than that predicted by in vitro experimentation.

Objective 7: To identify suitable donors for the establishment of humanised models for the profiling of test agents
We have tested multiple donors and established humanised models of different rates of human engraftment; one donor with highest engraftment was selected and used for profiling of a test agent.

4.4 For key scientific questions/objectives you could not complete either fully or partly, please include an explanation as to why you were not able to achieve what you set out to achieve when the licence was granted (reasons could for example include lack of funding, the model took longer than expected to develop, another group published data which meant continuing the work would equate to repeating the work, etc.).

The only objective that has not yet been completed is Objective 6, which we expect to complete before the end of the licence.

4.5 In the expected benefits section of your licence, you described the benefits you believed would be likely to arise from your programme of work. Please explain what benefits have actually been achieved and, if appropriate, explain how these differ from what you anticipated you could achieve when you wrote the licence.

This licence had the anticipated benefit of generating data essential for the development of new drugs. This benefit has been achieved, with the majority of objectives already completed with 14 months before the licence expires.

We have generated PK and biodistribution data which has enabled decisions to be made as to the best test agents to progress to testing in disease animal models as they are most likely to be successful drugs. Similarly, tolerability studies have directly fed into longer term studies in disease models (under other licences) as well as further studies on this licence investigating the effect of test agents on biomarkers. Tolerability studies have also allowed test agents with unacceptable side effects to be ruled out from further animal study work. In parallel, PK and tolerability studies have also allowed appropriate dosing regimens for future disease model studies to be determined.

Addition of the protocol to allow administration of human immune cells to immunocompromised mice has allowed us to test potential therapeutics which rely on the interaction with human immune cells to have their effect. Whilst those test agents tested through this protocol so far have not shown the required functionality to be progressed into further disease model studies, this has provided valuable information for the development of these test agents further to improve their action. These newer types of therapeutic are aiming to treat diseases such as cancer where current treatments are limited and have significant side effects, with the aim of providing treatment options with fewer unwanted side effects. Whilst there is not yet a test agent suitable for progression to disease model testing, the work we have performed has helped to progress the design of the test agents.

One of the key things we aim to do in our work is to determine at an early stage whether a test agent is likely to become a successful human drug. As part of the work to find test agents that can become human therapeutics, it is important to rule out drugs early in the developmental process if they are unlikely to be suitable for use in humans. Our PK and tolerability work in particular has provided data which has led to the decision for work using several test agents to be halted. This has ensured that

animals have not been used unnecessarily in larger disease model studies and has allowed resources to be focused on other test agents, ultimately increasing the likelihood of a successful new drug being developed.

The overarching goal and the long-term benefit of our work together with clients is to for new drugs to make it to the clinic for patients. One drug tested under this licence has now been dosed to humans in phase 1 clinical trials, and a clinical trial application has been submitted for another. We receive regular updates from our clients when the therapeutics move to later stages in clinical development. Currently, there are several other test agents initially investigated under this licence which have progressed to the next stage of development in disease models (under other licences) and for which clinical trial applications are expected to be submitted in the coming years.

4.6 Specifically, if you were unable to address any or even all your key scientific questions/objectives what impact did this have on the benefits arising from your work?

There is only one objective which has not yet been achieved (Objective 6, target engagement of at least 2 test agents), and we expect to complete this within the 14 months remaining on the licence.

4.7 Were you able to identify/develop/implement any new scientific or technical developments during the tenure of this licence? **YES** ~~NO~~

If so, what were these and what were/was the benefit(s)?

We have not developed any completely novel methods during this licence but have adopted and optimised in-house a variety of techniques and approaches that were new to the team. For example, intraportal delivery of test agents and establishment of humanised mouse models have been introduced as elaborate systems to test novel therapeutics.

We have tested over 90 novel test agents during this licence and expect to test several more over the remaining tenure of the licence. This work has advanced scientific knowledge of our clients for the benefit of patients. Some of these test agents tested are also newer technologies, meaning we have contributed to technical developments in therapeutic design, although the technical developments made in the test agents were performed by our clients.

4.8 Using the headings below please provide a list of publications and meetings attended where research arising from work undertaken under this licence has been disseminated.

Please highlight in bold the publications which contain and meetings at which 3Rs achievements were disseminated.
Please only include publications arising from research undertaken under the authority of this licence. If you have papers in press, please include these.

Publications in scientific journals: None

As we are a drug discovery service provider, we are usually bound by confidentiality agreements and/or need to maintain competitive advantage, therefore publications are rare. Our work is used in patents and regulatory filings where test agents progress through towards becoming new therapeutics.

Publications pending, or under review: Paper on the hock issues in NSG mice in preparation.

Presentations at scientific meetings: **Development of a monitoring and treatment regimen for NSG mice displaying phenotypic tarsal joint swelling and hock lesions – Poster at LASA conference, 2024**

Have you remembered to highlight in bold the publications which contain and meetings at which 3Rs achievements were disseminated?

4.9 Have you engaged with the general public by giving interviews about your work on radio, television, at a public meeting or debate or in a newspaper article? ~~YES~~ **NO**

If you answered NO: why?

Due to the nature of our work as a service provider in the drug discovery industry, we are bound by confidentiality agreements to ensure that our clients are able to maintain a competitive advantage. This means that it is difficult to fully engage in public activities. However, two members of our team have given talks to groups of drug discovery Master's students. one of these events is a yearly recurring lecture as part of the degree, the other was a career talk.

If you answered YES: how, where (i.e., what medium did you use) and when did you engage with the public, what sort of information did you disseminate and what was the outcome?

N/A

5. Project Licence management information.

5.1 Amendments (if applicable):
Provide dates and a summary of all amendments made to this licence (i.e., what each amendment requested and why it was required) since the licence was granted (or if appropriate since the licence was last reviewed).
Please read questions 2.11 and 2.12 – for amendments that apply to this question including those that apply to either 2.11 or 2.12, please provide the date of the amendment here and, if relevant, a note that further information can be found in Section 2.11 or 2.12.

Granted 25 April 2022

- Addition of minipump implantation to protocol 7
  - This was required to more closely mimic the expected route of administration for a particular test agent in humans (intravenous infusion).

Granted 28 September 2022

- Addition of a new objective (5) and protocol (8) for the investigation of the biodistribution of test agents
  - This was required to perform biodistribution investigations for a new client, following on from preliminary work they had performed elsewhere.
- Addition of a new protocol (9) for the investigation of the tolerability of test agents dosed continuously
  - As with the previous amendment, this was to allow us to dose compounds in a more clinically relevant setting.

Granted 9 March 2023

- Addition of a new protocol (10) for the delivery of test agents directly to the brain

    - This was required to test potential therapeutics for neurological disorders for a new client.
- Addition of collection of tissues under terminal anaesthesia to protocol 7.
    - This was required to allow the collection of CSF for an existing client
- Increase in the maximum number of mice on protocol 1.
    - This was required to provide samples for the optimisation of a biomarker assay.

Granted 16 June 2023

- Increase in the maximum number of rats on protocol 7.
    - This was required to cover an additional programme of work for an existing client to gather valuable information on a test agent to determine whether it could move into clinical trials.

Granted 8 November 2023

- Inclusion of liver failure as a new disease area
- Addition of a new protocol (11) for delivery of test agent to the liver via the portal vein..
    - These two parts were required to allow testing of potential therapeutics for liver failure for a new client.
- Clarification of test agent definition
    - This was required to ensure that the interpretation of 'test agent' within the licence was clear.

Granted 7 June 2024

- Clarification of wording for weight loss humane endpoints (protocols 2, 3, 5, 8, 9, 10, 11)
    - This was required as we had found that the previous wording was unclear in practice.
- Clarification for monitoring in tolerability studies (Protocol 6, step 1)
    - This was required to cover a word excluded in error from the original PPL. This change reduced the mandated monitoring period for tolerability studies where dosage of test agent is not altered, which reduced the requirement for unnecessary disturbance to the animals.
- Addition of standard NSG mice adverse effects wording (Protocols 5, 6, 7, 8, 11)
    - This was required to include the wording agreed by the University upon the recognition that nock issues in NSG mice are a regularly occurring issue.

Granted 31 October 2024

- Inclusion of a new objective (6) and protocol (12) for target engagement studies
    - This was required to perform studies for a new client who is developing novel 'cargo' based therapies.
- Inclusion of a new objective (7) and protocol (13) to determine suitable human donors of immune cells and to test the effect on biomarkers of test agents in humanised mice
    - This was required to allow the testing of drugs that interact with the human immune system in a more relevant setting, and also to allow testing of drugs for graft versus host disease.
- Addition of AB code to intraperitoneal dosing (protocol 7)
    - This was required for the testing of a cell-based therapy which required injection under anaesthesia using a larger needle due to the size of the cells and under conditions of greater control and slower speed than is possible in conscious mice.
- Addition of intramuscular dosing (Protocols 6 & 7)
    - This was required for the testing of cell-based therapies injected directly into the muscle.

Granted 31 October 2025

- Inclusion of a new method for administration of test agents to the liver (protocol 11)
    - This was required to allow delivery of test agents to the liver via an alternative, and potentially more effective route.

- Inclusion of a new protocol (14) for the administration of test agents to the kidney capsule.
    - This was required to allow testing using the kidney capsule to provide a 'pocket' for test agents to remain in and function in the body.
- Inclusion of subcutaneous fat pad injection to protocols 7 & 8.
    - This was to allow the testing of injection into the fat pad as a potential 'home' for cell-based treatments, as a less invasive route of administration than injection into the kidney capsule.
- Inclusion of imaging step to protocols 10, 11 & 14.
    - This was to allow collection of data on test agent migration in the body for an existing client who had developed a method of labelling their therapeutic cells with a luminescent tag.

Granted 18 March 2025

- Inclusion of new adverse effects for direct brain injection (protocol 10)
    - This was required following observation of partial hind limb paralysis following direct brain injection.

Granted 15 May 2025

- Inclusion of aged mice on protocols 1, 2, 7 & 8
    - This was required to allow work for two clients who were developing therapeutics for diseases of the aged population.

Granted 28 July 2025

- Addition of infectious diseases as a new disease area
    - This was required to perform work for multiple new clients who required test agent profiling (in healthy mice) of potential drugs for infectious diseases.
- Clarification of weight loss endpoints on protocol 8
    - This was required to correct a discrepancy between the reference point for the weight loss calculations within step 2 of protocol 8.

5.2 Project licence Standard Condition 18 Reports (if applicable):
Provide dates and copies of all Condition 18 reports sent to the Home Office since the licence was granted (or if appropriate since the licence was last reviewed). Copies of any reports should be attached/supplied as separate documents please.

Please see attached appendix containing details and copies of all SC18 reports.

5.3 Non-Compliance (if applicable):
Provide the date, a summary of the nature of any non-compliance and the outcome (or if appropriate since the licence was last reviewed). Include the RCA reference number.

2025-41: A mouse tail was trapped between the cage lid and the cage base overnight. The mouse was able to place all feet on the base of the loft. The mouse had lost 5.8% bodyweight overnight, compared to a loss of ~2% for other mice on the study. After release, the mouse was behaving normally and was not displaying any indication of pain. A dose of Metacam was administered upon advice from the NVS. The staff members involved in placing this cage back on the rack were reassessed for cage assembly.

2024-86 & 2024-92: Two rats were killed CD by staff members, who whilst having extensive previous experience, did not have a current competency assessment for this method of killing in rats. In both cases, the rats were experiencing an adverse effect due to an oral gavage dose being inadvertently

administered to the lung, and the CD method of killing was utilised quickly and efficiently to minimise the suffering of the animal. It was confirmed by an NVS that where a protected animal needs to be killed as a matter of urgency there is not a requirement for the person carrying out the killing to be on the establishment register. The team members who performed the oral gavage were re-trained/assessed for the oral gavage procedure.

5.4 Have there been any other issues that have affected the management of this licence that you feel should be taken into consideration, (e.g., equipment failure, absence of key staff, change in the health status of the animal unit etc.)?

None

5 Retrospective Review:

Project Licence Holder check list:

AWERB requires the following documents	Documents sent to AWERB
Completed copy of this form	
Copies of Condition 18 reports (if applicable)	