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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:

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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 (Madingley)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Mild
1. Date of incident	09/03/2023

2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to determine correct dose of doxycycline to give to induce expression of eIF6, producing a model of Shwachman-Diamond syndrome. This pilot study is to determine the correct genotype/dox dose combination to induce changes in blood parameters, in a “leapfrog” dose escalation design (please see attached study plan). 15% Ribena is given with the dox water to help palatability. Based on the literature we had anticipated modest transient weight loss not exceeding 10%. However, in initial studies weight loss has varied substantially and close to 10%, even with supportive care such as mash. The weight loss is random, not related to genotype but does sometimes correlate to the initial stages of a dox dose increase. At this stage while dose is determined, dox is being given in the drinking water (as opposed to fixed dose dox food) and this may contribute to the weight loss being seen. We have seen fluctuations in weight of 5% from day to day, so we think it is variable water intake/hydration in most cases and not true body weight change. **Based on the first pilot study an amendment to seek permission to allow 15% weight loss was submitted locally mid-January, with AWERB yesterday (08/03/23), so as soon as comments are back we will submit this.** Today (09/03/23) one mouse has had a weight loss of 12.5%, only reducing to 11.4% after being given mash which is beyond the humane endpoint. This animal and any others that have shown 10% weight loss have not displayed any clinical signs; they have had a healthy appearance, are alert and gained weight. The breeding of these animals is complex, and the pilot studies are giving us important information to induce the desired changes in blood parameters (reticulocytes primarily) without clinical signs, so these animals are very valuable scientifically.

	We ask that we keep the cohort of animals in this study alive to a new humane endpoint of 15% BW loss. If permitted, any animals that exceed 15% body weight loss will be killed (or additional HO advice sought).
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	NACWO and NVS have been informed and sent a draft of the report.
5. Cause of Problem, if known	
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)*	Animals will continue to be monitored daily and given supportive care on study. Following discussions regarding the amendment at AWERB, a central record of different methods/substances to improve palatability is to be set up at the University to aid all researchers.
7. Reported by (name)	Amy Warner
8. Date	09/03/2023

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	

Date	

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Establishment Licence name	University of Cambridge (Madingley)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Mild
1. Date of incident	29/03/2023

2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to determine correct dose of doxycycline to give to induce expression of eIF6, producing a model of Shwachman-Diamond syndrome. This pilot study is to administer a fixed doxycycline dose in a commercially available chow, to various genotypes of the SDS model. Initial studies were using dox in the drinking water, but as soon as a suitable dose was found we moved to diet. In the drinking studies we had animals that were losing around 10%, as we believe there are some phenotypic weight loss effects. The weight loss is random to a certain extent though, with some animals of the same genotypes having very different weight profiles. **Based on the first pilot study an amendment to seek permission to allow 15% weight loss was submitted locally mid-January, AWERB mid-March, and is currently with the HO.** The study giving dox-chow started on 20/03/23. Today (29/03/23) one mouse (ID 6) had a weight loss of 9.5% at the start of the day, and by the end of today (4:30pm) was down to 12.7% which is beyond the humane endpoint. This animal was given (dox) mash in the day but did not show much interest. This animal is not displayed any clinical signs; it has a healthy appearance and is alert. The animals BW loss percentages for the last 6 days have been -6.9, -7.8, -8.2, -9.5, -8.2, -9.5 respectively. While we wait to hear back the mouse has been put into a separate cage and given normal chow mash overnight in an attempt to see if it is aversion to the dox diet (no other animals are showing this), or possibly a dominance problem with a cagemate. The breeding of these animals is complex, and the pilot studies are giving us important information to induce the desired changes in blood parameters (reticulocytes

	primarily) without clinical signs, so these animals are very valuable scientifically. **We ask that we keep this animal alive in this study alive to a new humane endpoint of 15% BW loss, while we wait for news of the amendment.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, with no clinical signs, the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	
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)*	Animals will continue to be monitored daily and given supportive care on study.
7. Reported by (name)	Amy Warner
8. Date	29/03/2023

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Inspector's comments and recommendations:

Comments and recommendations	Have read and noted the above. Agree cause of weight loss may be diet aversion related or a dominance issue. Authority to keep this mouse alive is granted subject to the following conditions: 1. This is supported by the NVS. 2. The amendment increasing the HEP to 15% by 13 Apr 23 or the mouse's weight has returned to less than 10% weight loss. 3. Its weight loss does not exceed 15%.
Inspector name	Neil Smith
Date	30 Mar 23

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	16/06/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to administer a novel therapeutic agent twice daily via intraperitoneal injection for 2 weeks to mice of various genotypes of the SDS model that have been treated with doxycycline in a commercially available chow to examine tolerability (in the model animals) and the potential efficacy of the novel therapeutic agent on various end points that are altered in the SDS model. The dose of the novel therapeutic agent will be escalated if tolerability is able to be demonstrated. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days has been observed in animals of various genotypes of the SDS model when treated with the doxycycline diet. The study giving dox-chow and administering the novel therapeutic agent started on 08/06/23. Today (16/06/23) one mouse (ID 8) had a weight loss of 15.9% from its peak bodyweight, which is beyond the humane endpoint of a 15% body weight loss from peak study weight sustained over 2 consecutive days. The animal was provided with (dox) wet mash from 13/06/2023 as a supportive measure. The mouse is not displaying any clinical signs; it has a healthy appearance and is alert. The animals BW loss percentages for the previous 3 days have been -12.7, -14.0, -13.7 respectively. While we wait to hear back the mouse has been given a break from dosing and provided with additional dietary supplementation in the form of cheerios and trail mix. If no improvement is seen in this animal's bodyweight by the end of the working day it will be separated from it's cage mate and provided with normal chow mash overnight.

	The breeding of these animals is complex, and the initial studies with novel therapeutic agents are giving us important information to examine the effects of the novel test agent on changes in blood parameters (reticulocytes primarily) without clinical signs, so these animals are very valuable scientifically. **We ask that we keep this animal alive for a further 48 hours to examine whether the measures described above are able to return the animal to within the permitted humane endpoint and allow it to continue on study to provide valuable data.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, with no clinical signs, the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	
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)*	Animals will continue to be monitored daily and given supportive care on study.
7. Reported by (name)	Robert Carrington
8. Date	16/06/2023

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Inspector's comments and recommendations:

Comments and recommendations	Thank you for this very clear and prompt PPL SC18 report, which I confirm needed to be made. Your proposed approach of allowing 48 hours for the animal to recover to less than the 15% weight loss endpoint is acceptable, with the following caveats: • If weight loss continues and reaches 20% or more in this 48-hour period, the animal should be immediately killed • If the animal's weight loss is still over the 15% endpoint at the end of this 48-hour period, the animal should be killed.
Inspector name	Dr Rick Carver
Date	16 Jun 2023

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	11/08/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to determine suitability of mice that are homozygous for both the doxycycline response element and the eIF6 gene for future studies by treating these animals with doxycycline in a commercially available chow to examine the phenotype produced and compare to data from pilot studies that utilised mice with genotypes of mixed heterozygous and homozygous expression of the 2 genes of interest. If tolerability of gene induction is demonstrated and a sufficient disease phenotype has been induced (as determined by changes in blood parameters such as reticulocyte numbers and haemoglobin levels) then these animals may also be administered a novel therapeutic agent to determine both potential tolerability and efficacy. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days has been observed in animals of various genotypes of the SDS model when treated with the doxycycline diet. Bringing about the disease state based on ex vivo data has so far been sub-optimal in heterozygotes, so this study is to determine whether using homozygotes can increase the therapeutic window. The study giving dox-chow and administering the novel therapeutic agent started on 01/08/23. Today (11/08/23) one mouse (ID 4) had a weight loss of 16.5% from its peak bodyweight which is beyond the humane endpoint of a 15% body weight loss from peak study weight sustained over 2 consecutive days. The animal was provided with (dox) wet mash, normal chow mash and treats in various combinations throughout the last few days as a supportive measure. The mouse is not displaying any clinical signs; it has a healthy appearance and is alert.

	The animals BW loss percentages for the last 3 days have been -11.5%, -6.9%, -14.2% respectively. Taken together with the other homozygous mouse reported earlier in the week (07/08), we do not anticipate that we will use the combination of homozygous animals and 625mg/kg dox diet, opting for different genotypes or a lower dox concentration. We will humanely kill this animal as we are unable to keep it above the humane end point whilst still maintaining an adequate dox dose. However, this animal could provide valuable information if allowed to stay alive until later today where we can analyse blood parameters via flow cytometry in the first available slot. This would allow us to examine the effects of this level of gene induction and potentially novel test agent on changes in blood parameters (reticulocytes primarily) without clinical signs, so this animal is still very valuable scientifically. We will give it supportive care in the meantime. **We ask that we keep this animal alive until 4pm today (11/08) in order to capture valuable data from this animal.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, with no clinical signs, the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	With a pilot cohort of 2 animals it is hard to determine definitively, but we suspect that the combination of genotype and dox dose is causing the weight loss rather than external factors.

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)*	This animal is the last of its kind on this study.
7. Reported by (name)	Amy Warner
8. Date	11/08/2023

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Inspector's comments and recommendations:

Comments and recommendations	Given that: • this animal is showing no adverse effects other than the additional body weight loss • the requested time to keep this animal alive is very short (and well within the maximum 14-day period that an Inspector is authorised to allow) • keeping this animal alive could yield useful data • therefore, this request achieves a favourable HBA. I am content for this animal to be kept alive until 16:00 hrs on Fri 11 Aug 2023.
Inspector name	Dr Rick Carver
Date	11 Aug 2023

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	12/10/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to examine the dose response to doxycycline of mice that are heterozygous for both the doxycycline response element and the eIF6 gene and in animals that are heterozygous for the doxycycline response element and homozygous for the eIF6 gene and to determine whether there is a therapeutic window in this model. This is being done by dosing animals with a novel therapeutic agent for 1 week and then beginning doxycycline administration at a number of different dose levels in the drinking water (combined with Ribena to attempt to mask any bitterness from the additional of doxycycline) while continuing dosing with the novel therapeutic agent. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days was observed in animals of various genotypes of the SDS model when treated with doxycycline in the diet and drinking water, and repeat dosing of similar therapeutic agents has previously been tolerated in doxycycline treated animals of various genotypes. Since the pilot most animals on study have at some point been between 10-15% while we bring about a disease state sufficient for studying potential drugs. The therapeutic window based on ex vivo data has so far been sub-optimal, so this study continues to determine whether using alternative doses of doxycycline can induce a robust disease state while being tolerated by the animals and still having the potential to be modulated by prophylactic therapeutic agents. The study administering the novel therapeutic agent started on 04/10/23 with doxycycline added to drinking water on 11/10/23 for cohort 1 and a second cohort beginning 1 day later.

	Today (12/10/23) one mouse (ID 4) had a weight loss of 18.2% from its peak bodyweight and a second mouse (ID16) had a weight loss of 18.6% from its peak bodyweight which is beyond the humane endpoint of a 15% body weight loss from peak study weight sustained over 2 consecutive days. The animals were provided with wet mash, and treats in various combinations throughout the last few days as a supportive measure. The mice were not displaying any clinical signs; and had a healthy appearance and were alert. Both of these animals were in cohort 1 and in a group receiving the novel therapeutic agent. The animals BW loss percentages for the last 3 days have been -12.6%, -12.8%, and -13.8% for mouse 4 and -10.9%, -11.8% and -14.6% for mouse 16 respectively. Both mice were due to have blood samples taken from the lateral tail vein yesterday (11/10/23) but in an attempt to minimise any stress that could induce further weight loss these procedures were not carried out for these specific mice. These animals were sch1 killed immediately and terminal samples taken.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO and NVS have been sent this report alongside submission.

5. Cause of Problem, if known	It is possible that the weight loss could be connected to therapeutic agent treatment, however there are a number of animals in the same treatment group that have not exhibited the same level of weight loss. The sudden drop of 4.4 % and 4% for mice 4 and 16 respectively overnight is likely to be due in part to aversion to their drinking water with the addition of doxycycline yesterday which has been attempted to be mitigated with the combination of drinking water with Ribena to mask the taste of doxycycline, along with an acclimatisation period of 1 week to Ribena in the drinking water without doxycycline to attempt to familiarise the animals to the taste of Ribena prior to adding doxycycline.
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)*	The animals that exceeded the weight loss limits have been sch 1 killed and terminal samples taken. Supportive measures in the form of wet mash and treats are being provided to all other animals on study.
7. Reported by (name)	Amy Warner
8. Date	12/10/2023

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Inspector’s comments and recommendations:

Comments and recommendations	Thank you for the PPL SC18 Report. You are compliant with the licence conditions by reporting this under PPL Standard Condition 18. The content of this SC18 has been read and noted and can now be considered CLOSED. Please do inform us of any other related incidents should they occur. Date 18/12/23

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Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	13/10/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to examine the dose response to doxycycline of mice that are heterozygous for both the doxycycline response element and the eIF6 gene and in animals that are heterozygous for the doxycycline response element and homozygous for the eIF6 gene and to determine whether there is a therapeutic window in this model. This is being done by dosing animals with a novel therapeutic agent for 1 week and then beginning doxycycline administration at a number of different dose levels in the drinking water (combined with Ribena to attempt to mask any bitterness from the additional of doxycycline) while continuing dosing with the novel therapeutic agent. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days was observed in animals of various genotypes of the SDS model when treated with doxycycline in the diet and drinking water, and repeat dosing of similar therapeutic agents has previously been tolerated in doxycycline treated animals of various genotypes. Since the pilot most animals on study have at some point been between 10-15% while we bring about a disease state sufficient for studying potential drugs. The therapeutic window based on ex vivo data has so far been sub-optimal, so this study continues to determine whether using alternative doses of doxycycline can induce a robust disease state while being tolerated by the animals and still having the potential to be modulated by prophylactic therapeutic agents. The study administering the novel therapeutic agent started on 04/10/23 with doxycycline added to drinking water on 11/10/23 for cohort 1 and a second cohort beginning 1 day later. Yesterday (12/10/23) one mouse (ID 4) had a weight loss of 18.2% from its peak bodyweight and a second mouse

	(ID16) had a weight loss of 18.6% from its peak bodyweight which is beyond the humane endpoint of a 15% body weight loss from peak study weight sustained over 2 consecutive days, but showed no clinical signs. These were sch1 killed immediately, and reported on (SC18 sent 12/10/23). Today four further animals have exceeded the humane endpoint, but remain alert with no clinical signs. Body weight loss for each animal over the last 3 days is below (today's BWL in bold): ID 2: -10.5% -11.3%, -**16.5%** ID 43:-12.7%, -11.6%, **-17.4%** ID 46:-9.2% -10.8%, **-16.3%** ID 48:-9.0% -9.5%, **-16.2%** **We ask that we keep these 4 animals alive for up to 10 days with a body weight loss limit of up to 20%, with no clinical signs. Any animals that exceed 20%, or have clinical signs, would be promptly sch1 killed.** **If possible, we would also ask for this to be extended to all animals on study (54 mice), while we try to optimise supportive care, and secure a therapeutic window. These mice are giving us valuable information on a range of factors that will inform on the next study, and are precious genotype combinations.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, with no clinical signs, the NACWO and NVS have been sent this report alongside submission.

5. Cause of Problem, if known	As these animals are a mix of vehicle- and test agent-treated, we don't believe it is pharmacologically driven. The vehicle substance (5% DMSO in corn oil) should not be causing this, but we will halve the dosing volume (5 ml/kg to 2.5 ml/kg) to lessen any potential effects. Body weights in general on this study has been very variable; it could be due in part that the animals are older and so more likely to be stressed by daily dosing. As with previous studies it could also be due in part to aversion to their drinking water with the addition of doxycycline. Ribena has been used 1 week before dox was introduced to mask the taste, however to does not seem to mitigate completely.
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)*	We have taken the following steps today: • A dosing break for today for the 4 mice. • Introducing Complan (high nutrient supplement) for mice that are over 12% BWL. • Halving the dose volume for all animals from tomorrow (14/10/23).
7. Reported by (name)	Amy Warner
8. Date	13/10/2023

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	17/10/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to examine the dose response to doxycycline of mice that are heterozygous for both the doxycycline response element and the eIF6 gene and in animals that are heterozygous for the doxycycline response element and homozygous for the eIF6 gene and to determine whether there is a therapeutic window in this model. This is being done by dosing animals with a novel therapeutic agent for 1 week and then beginning doxycycline administration at a number of different dose levels in the drinking water (combined with Ribena to attempt to mask any bitterness from the additional of doxycycline) while continuing dosing with the novel therapeutic agent. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days was observed in animals of various genotypes of the SDS model when treated with doxycycline in the diet and drinking water, and repeat dosing of similar therapeutic agents has previously been tolerated in doxycycline treated animals of various genotypes. Since the pilot most animals on study have at some point been between 10-15% while we bring about a disease state sufficient for studying potential drugs. The therapeutic window based on ex vivo data has so far been sub-optimal, so this study continues to determine whether using alternative doses of doxycycline can induce a robust disease state while being tolerated by the animals and still having the potential to be modulated by prophylactic therapeutic agents. The study administering the novel therapeutic agent started on 04/10/23 with doxycycline added to drinking water on 11/10/23 for cohort 1 and a second cohort beginning 1 day later. On 13/10/23 a SC18 report was submitted to request to keep alive 4 specific animals that had exceeded the

	weight loss limits on the licence, and also requested that authority be given for all animals on study to keep alive any other animals on study that exceeded 15% weight loss, but no greater than 20% weight loss that had no associated clinical signs. While awaiting a response all animals that exceeded 15% but no greater than 20% weight loss were kept alive and daily emails were sent to the compliance team to update on these animals. On 17/10/23 it was communicated that authority could be granted for the 4 specific animals detailed in the report that was submitted on 13/10/23 but that authority could not be granted for other animals on the study. Therefore this report details the animals that were not specifically detailed in the SC18 report on 13/10/23 that exceeded the 15% weight loss limits while awaiting a response to the SC18 report. The following mice were not specified on the original SC18 and exceeded the 15% weight loss limits. Body weight loss for each animal over the last 4 days is below (today's BWL in bold):: ID 8 -14.1%, -16.6%, -16.6%, **-10.9%** ID 11 -15.1%, -19.8%, -21.5% Animal was immediately culled having exceeded 20% and showed no clinical signs. ID 17 -13.6%, -17.8%, -15.7%, **-7.4%** ID 20 -12.7%, -13.5%, -16.0%, **-9.8%** ID 36 -12.2%, -16.3%, -16.3%, **-8.6%** ID 44 -19.1%, -9.2%, -9.2%, **-11.5%** ID 53-16.2%, -22.8% Animal was immediately culled having exceeded 20% and showed no clinical signs.

	As mice 8, 17,20,36, and 44 have all returned to within 15% weight loss limits we will continue with them on study and submit further SC18 reports if required.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO and NVS were sent the original report submitted on 13/10/23 and have been included on the updates provided to the home office since this submission.
5. Cause of Problem, if known	As these animals are a mix of vehicle- and test agent-treated, we don't believe it is pharmacologically driven. The vehicle substance (5% DMSO in corn oil) should not be causing this, but we have halved the dosing volume (5 ml/kg to 2.5 ml/kg) to lessen any potential effects. Body weights in general on this study have been very variable; it could be due in part that the animals are older and so more likely to be stressed by daily dosing. As with previous studies it could also be due in part to aversion to their drinking water with the addition of doxycycline. Ribena has been used 1 week before dox was introduced to mask the taste, however to does not seem to mitigate completely.
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)*	We have taken the following steps: • Reducing dose volume from 14/10/23. • Enhanced dietary supplementation in the form of Complan (high nutrient supplement) for all mice. • A dosing and doxycycline break for all animals since yesterday (16/10/23) while continuing all dietary supplementation. As advised by email we plan to resume the study, likely reduced in some capacity (e.g. lower dox dose, reduced dosing etc) to obtain the maximum amount of data possible from these animals
7. Reported by (name)	Rob Carrington
8. Date	17/10/2023

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Establishment Licence name	University of Cambridge (Madingley)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	17/10/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to examine the dose response to doxycycline of mice that are heterozygous for both the doxycycline response element and the eIF6 gene and in animals that are heterozygous for the doxycycline response element and homozygous for the eIF6 gene and to determine whether there is a therapeutic window in this model. This is being done by dosing animals with a novel therapeutic agent for 1 week and then beginning doxycycline administration at a number of different dose levels in the drinking water (combined with Ribena to attempt to mask any bitterness from the additional of doxycycline) while continuing dosing with the novel therapeutic agent. In pilot studies a moderate level of weight loss (around 10 %) with recovery after approximately 7-10 days was observed in animals of various genotypes of the SDS model when treated with doxycycline in the diet and drinking water, and repeat dosing of similar therapeutic agents has previously been tolerated in doxycycline treated animals of various genotypes. Since the pilot most animals on study have at some point been between 10-15% while we bring about a disease state sufficient for studying potential drugs. The therapeutic window based on ex vivo data has so far been sub-optimal, so this study continues to determine whether using alternative doses of doxycycline can induce a robust disease state while being tolerated by the animals and still having the potential to be modulated by prophylactic therapeutic agents. The study administering the novel therapeutic agent started on 04/10/23 with doxycycline added to drinking water on 11/10/23 for cohort 1 and a second cohort beginning 1 day later. As we informed in update emails regarding the previous keep alive request for this study submitted on 13/10/23, yesterday (16/10/23) animals

	were given a break from both doxycycline and test item administration, and now all but 2 animals are within the weight loss limits (one mouse, ID 43 is covered by the previous SC18 keep alive request). Today (17/10/23) one mouse (ID 38) had a weight loss of 15.6% from its peak bodyweight with no associated clinical signs. Body weight loss for this animal over the last 3 days is below (today's BWL in bold): ID 38: -13.6% -14.4%, -**15.6%** **We ask that we keep this animal alive for up to 14 days with a body weight loss limit of up to 20%, with no clinical signs. If this animal exceeds 20%, or exhibits clinical signs, it will be promptly sch1 killed.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO and NVS were sent the original report submitted on 13/10/23 and have been included on the updates provided to the home office since this submission.

5. Cause of Problem, if known	As these animals are a mix of vehicle- and test agent-treated, we don't believe it is pharmacologically driven. The vehicle substance (5% DMSO in corn oil) should not be causing this, but we have halved the dosing volume (5 ml/kg to 2.5 ml/kg) to lessen any potential effects. Body weights in general on this study have been very variable; it could be due in part that the animals are older and so more likely to be stressed by daily dosing. As with previous studies it could also be due in part to aversion to their drinking water with the addition of doxycycline. Ribena has been used 1 week before dox was introduced to mask the taste, however to does not seem to mitigate completely.
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)*	We have taken the following steps: • Reducing dose volume from 14/10/23. • Enhanced dietary supplementation in the form of Complan (high nutrient supplement) for all mice. • A dosing and doxycycline break for all animals yesterday (16/10/23) while continuing all dietary supplementation. As advised by email we plan to resume the study, likely reduced in some capacity (e.g. lower dox dose, reduced dosing etc) to obtain the maximum amount of data possible from these animals, later today.
7. Reported by (name)	Amy Warner
8. Date	17/10/2023

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Comments and recommendations	

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Establishment Licence name	University of Cambridge (Madingley)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	13/06/2023

2. Species	Mouse
3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline induced expression of eIF6. The aim of this study is to administer a novel therapeutic agent twice daily via intraperitoneal injection for 2 weeks to mice of various genotypes of the SDS model that have been treated with doxycycline in a commercially available chow to examine tolerability (in the model animals) and the potential efficacy of the novel therapeutic agent on various end points that are altered in the SDS model. The study giving dox-chow and administering the novel therapeutic agent started on 08/06/23. Today (13/06/23) all of the mice were dosed in the morning with no issue (as with on all other occasions). During the afternoon dosing one mouse (ID 22) was dosed as the last in its cage; the handling and dosing went fine, but upon getting ready to place the mouse back in the cage it stiffened. The researcher placed the mouse back into the empty home cage to observe (all cagemates were in a holding cage). Within seconds the mouse stretched their hind legs and stiffened further and went into what looked like a tonic-clonic seizure and fell onto its side. The mouse could not right itself and was somewhat unresponsive. The researcher immediately took the decision to sch1 kill the mouse by cervical dislocation due to its distress.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	As the mouse was immediately sch1 killed we did not seek advice, but they are copied into the sending of this report.

5. Cause of Problem, if known	We can rule out effect of test agent, as this mouse was being dosed with vehicle (corn oil/5% DMSO). A post-mortem examination showed no obvious signs of internal irregularities, with an intact gut ruling out mis-dosing. This mouse was on a doxycycline diet; although we have not seen any clinical signs related to this in this or any preceding studies. We suspect this was a rare neurological reaction to dosing that cannot be mitigated against, although we are very open to advice from our NVS or others.
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)*	We will continue to monitor animals on study for any early signs that this may happen again, and we will engage in a discussion with our NVS to see if there is anything further we can do.
7. Reported by (name)	Amy Warner
8. Date	13/06/2023

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	8
Protocol title	Inducible animal models of Shwachman-Diamond Syndrome
Severity classification	Moderate
1. Date of incident	18/06/2023

2. Species	Mouse
3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of Shwachman-Diamond syndrome (SDS) using doxycycline-induced expression of eIF6. The aim of this study is to administer a novel therapeutic agent twice daily via intraperitoneal injection for 2 weeks to mice of various genotypes of the SDS model that have been treated with doxycycline in a commercially available chow, to examine tolerability (in the model animals) and the potential efficacy of the novel therapeutic agent on various end points that are altered in the SDS model. The study giving dox-chow and administering the novel therapeutic agent started on 08/06/23. Yesterday (18/06/23) all of the mice were dosed in the morning with no issue. During the afternoon dosing one mouse (ID 12) was dosed; the handling and dosing went fine, but when withdrawing the needle the mouse stiffened. The researcher placed the mouse into an empty holding container to observe. The mouse stretched their hind legs and stiffened further and went into what looked like a tonic-clonic seizure and fell onto its side. The mouse could not right itself and was unresponsive. The researcher immediately took the decision to sch1 kill the mouse by cervical dislocation due to its distress. This all occurred within approx. 1 minute. This is virtually identical to the incident previously reported on this study on 13/06/2023, the only difference being that this mouse was dosed with test agent, not vehicle.

4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	As the mouse was immediately sch1 killed we did not seek advice at the time, but they are copied into the sending of this report. We have so far not had any comment from an NVS regarding the previous incident, but we will be in contact with them again.
5. Cause of Problem, if known	This mouse was dosed with the test agent; as the last mouse was treated with vehicle, we think it is unlikely to be the cause. A post-mortem examination showed no obvious signs of internal irregularities, with an intact gut ruling out mis-dosing. This mouse was on a doxycycline diet; although we have not seen any related clinical signs, in this or any preceding studies. This is the second mouse in the study for this to occur with, although we still believe this is a rare reaction to dosing as we’ve not seen it occur before this study.
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)*	We have researched the literature to see if there are any similar occurrences or commonalities we can draw from but have so far not found anything to explain this. We will continue to monitor animals on study for any early signs that this may happen again, although so far it has seemed impossible to predict as there are no preceding signs. The mice were littermates so we will monitor all related mice closely. The study will be terminated on Thursday (22^{nd}).
7. Reported by (name)	Amy Warner
8. Date	19/06/2023

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	30/09/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses wildtype animals to gain control data for the disease model using varying concentrations of plasmid to induce expression of a pro-inflammatory agent. This study forms part of a wider programme investigating human *APOL1*-associated kidney disease. 30 mice (15 males and 15 females) were included in this study. They were dosed with five different concentrations of vector ranging from 0.05 – 2 µg, or vehicle control. This vector induces overexpression of a pro-inflammatory agent (IFNγ) when administered by hydrodynamic injection. Mice were administered with the vector by hydrodynamic injection on Wednesday 27/09/23. Due to the failure of some injections, each dose was administered to 3-4 mice. Mice recovered well from the hydrodynamic injection procedure. Mice were provided with hydrogel and seeds. Mice were weighed on Thursday 28/09/23. Some small body weight loss was observed, as expected after anaesthesia and a hydrodynamic injection procedure. Mice were weighed again on Friday 29/09/23, where weight loss was found to have increased. All mice were well, displaying no clinical signs. Mice were provided with diet gel and seeds. Over the weekend (Saturday - 30/09/23), mouse 19 (female) was found to be displaying clinical signs (hunched) and was displaying body weight loss of -19.8% from the day of injection. She was immediately culled. This mouse was from the top dose (2 µg) dose group. Upon weighing of the remaining mice, body weight loss of greater than 15% was also observed in another 6 mice, however these 6 mice all appeared well and were not displaying clinical signs. Two of the mice (mouse 1, -16.4% and mouse 13, -18.1%), also from the top dose were culled as this has been deemed an unsuitable dose

	(Their weights leading up to this were: mouse 1 - -4.2%, -12.6%; mouse 13 - -3.2%, -10.9%; mouse 19 –4.6%, -14.2%). Note: A SC18 request to keep alive was submitted on Saturday for 4 of the mice on a lower dose that would provide vital information. This SC18 is to report the three mice that exceeded the 15% body weight loss limit and were immediately sch1 killed.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	As the animals were sch1 killed at the weekend with no further welfare concerns the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	
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)*	The remaining mice have been provided with diet gel and seeds. Body weight and mouse condition will continue to be monitored regularly, and we will await decision from the Keep Alive request for the remaining four mice. Information gained from this study will provide valuable information to feed into future study design. This may include adjustment of the vector dosage, or submission of an amendment to allow for greater weight loss without clinical signs if the lower doses of vector are found to be insufficient for the disease model.
7. Reported by (name)	Amy Warner
8. Date	02/10/2023

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	07/09/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 44 mice on the study, split across 2 cohorts. Yesterday (07/09/23), 18 heterozygous mice (cohort 1) were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Three different vectors were used containing control sequence or two different variations of coding sequence for IFNγ. Each vector was administered at 3 different doses, giving a total of 9 experimental groups. For each group, 1 male and 1 female was injected in this first cohort. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. A SC18 Keep Alive request was submitted for issues related to subdued behaviour. Unknown to the study director and PPL holder upon submitting this, 2 of the 18 mice could not be resuscitated from anaesthesia and died. This was communicated later in the day, in a gap in monitoring the subdued mice.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	We will fully discuss this with both the NACWO and NVS before doing any more hydrodynamic injections, after reviewing the data.

5. Cause of Problem, if known	In previous studies we have always performed hydrodynamic injections in conscious animals; as a group we have only lost one animal at the time of injection previously (total of 65 injections performed). There was a drive towards only performing HDI under anaesthesia, so we have switched, this being the first study. Although we were aware from other groups of some losses under anaesthesia during HDI we were hoping that our previous experience for the technique and refinements we had in place would mitigate the risk, but this is not the case. We will therefore be seeking an amendment to include the risk of death prior to dosing of the next cohort of animals.
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)*	A complete review of the full study and the test agent itself will be performed before cohort 2 is dosed. It is likely that a lower dosage will be used for the second cohort (relating to the subdued behaviour). However, there are no further refinements that can be made to the procedure itself to avoid the acute reaction which caused the deaths on this occasion.
7. Reported by (name)	Rachel Watson
8. Date	08/09/2023

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	07/09/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 44 mice on the study, split across 2 cohorts. Today (07/09/23), 18 heterozygous mice (cohort 1) have been administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Three different vectors have been used containing control sequence or two different variations of coding sequence for IFNγ. Each vector was administered at 3 different doses, giving a total of 9 experimental groups. For each group, 1 male and 1 female was injected in this first cohort. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. The mice all woke from the anaesthesia around 2 minutes after injection and became more active 10 minutes after injection. However, from around 40 minutes post-injection behaviour again became more subdued. 2 hours post-dosing, the mice remain subdued, displaying some piloerection. The effects observed are consistent for all the mice dosed, with no differences between mice dosed with different vectors or dosages. There are indications that the activity of mice who were dosed first is higher than those who were dosed later in the morning. The mice have been provided with hydrogel, diet mash and seeds and heat pads have been added to the cage.

	The humane end point within the PPL for this step is that mice who continue to display subdued behaviour 2 hours post-injection will be culled by a Schedule 1 method. When previously performing hydrodynamic injections, we had observed that mice had recovered from the procedure within this timeframe. However, all our previous hydrodynamic work had been performed in conscious mice, not under anaesthesia. We therefore suspect that the addition of anaesthesia has slowed the recovery from the procedure. **We ask that we keep this animal until 5pm today to monitor behaviour and determine whether the mice return to normal levels of activity. Any mice that do not recover by 5pm will be culled by a Schedule 1 method. These animals are scientifically precious, and this will provide vital information to feed into future study design.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	
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)*	The mice have been provided with hydrogel, diet gel and seeds and heat pads have been added to the cage. Animals will continue to be monitored closely until they return to normal behaviour or until Schedule 1 at 5pm, as appropriate. Information gained from the extended monitoring period will feed into future study design. If mice recover well within a slightly longer time frame, a PPL amendment will be submitted, as the nature of hydrodynamic gene delivery is such that the dose volume cannot be altered significantly.
7. Reported by (name)	Rachel Watson

8. Date	07/09/2023

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Inspector’s comments and recommendations:

Comments and recommendations	Thank you for your report of adverse effects unexpectedly continuing beyond the time stated in the licence. By reporting this you are compliant with PPL standard condition 18. This has been given case ID 231962 You have requested the animals be kept alive until at least 5pm today. If they have not fully recovered, they will be humanely killed. The animals are displaying signs of subdued behaviour. The named persons have been made aware of this incident and request to keep animals alive until 5pm. Since the animals have undergone a procedure (harms) if they were to be culled now, they would not accrue benefit and be wasted. Given the nature of the adverse effects, that animals which received the hydrodynamic delivery earlier in the day appear to be recovering and that you have put in place measures to mitigate the animals' response, I am content for the animals to remain alive until 5pm provided they continue to make good progress and not deteriorate further. Any animals not fully recovered by 5pm must be humanely killed. Following review of this incident you may wish to consider amending the project licence to take into account this extended recovery time, depending on the outcome of today's events.
Inspector name	Dr Peter Thornton ASRU Licensing
Date	07/09/2023

Home Office

PPL Standard Condition 18 Notification Form

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	19/02/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). This study involves administration of vectors for the overexpression of IFNγ by hydrodynamic tail vein injection (HTVI). Several studies have been performed using varying doses of vector (0.05 – 5 ug) to validate the disease model in heterozygous mice. A dose of 0.2 ug vector was determined to be most suitable for future studies, however, it was determined that studies needed to move into homozygous mice to generate the required disease phenotype. 22 mice (7 males and 15 females) were included in this study. They were dosed with 0.2 ug of either a vector encoding IFNγ (n=17) or the empty vector (n=5) on Friday 16th February. Four mice were removed from study during the first 24 hours due to reaching the humane end point as they did not recover well from the HTVI procedure, which was within expectations for this procedure. Today (Monday 19th February, study day 3), three mice are exhibiting weight loss of greater than 15%. The humane end point within the PPL is that mice who display body weight loss of 15% sustained over 2 consecutive days will be killed. The weight loss for these mice since HTVI is as follows: Mouse 3: -6.3%, -14.8%, -16.5% Mouse 8: -5.6%, -13.8%, -15.3% Mouse 18: -8.5%, -14.5%, -17.9% Mice 3 and 8 are both well, displaying no clinical signs. The NACWO is happy for both these mice to continue on

	study. Mouse 18 is showing occasional intermittent hunching, but is alert and active, showing normal mouse foraging behaviours in the cage. The NACWO has reviewed this mouse and has suggested separating him with fresh supplements and monitoring weight and behaviour after 2 hours, to determine if there has been any improvement. In all cases the rate of weight loss has slowed, and we have observed a stabilisation and recovery of weight loss in several other mice on study from study day 2 to study day 3. This matches the pattern observed in the previous study in heterozygous mice where weight loss stabilised from study day 2 to 3 and mice began to then regain some of the weight lost. The mice have been provided with supplements (seeds, complan) throughout the study and these are being refreshed regularly. We wish to request to keep alive these mice up to a maximum of 20% weight loss without associated clinical signs. For mouse 18, this would be subject to review by the NACWO at 1pm on Monday 19th February to confirm that the mouse is gaining weight and is suitable to remain on study having been separated from its cage mate and provided with fresh supplements. The mice are due to remain on study for a further 7 days (until Monday 26 February), with dosing of therapeutics commencing on Wednesday 21st February. The data gained from these mice will provide vital information regarding the disease model in homozygous mice and the potential efficacy of a novel therapeutic and will feed into future study design.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO has observed the mice and is happy with our proposal to keep mice 3 & 8 alive. She has suggested a plan with a review point at 1pm for mouse 18, as described above.

5. Cause of Problem, if known	The weight loss is due to a combination of the expression of IFNγ and likely the subsequent increase in APOL1 expression and renal phenotype in the mice. Mice in this study dosed with empty vector are displaying much lower levels of weight loss, with some of this group now recovered back to starting body weight from the HTVI. The weight loss observed in these mice is greater than that observed in heterozygous mice, indicating that the renal phenotype caused by increased APOL1 expression is playing a part in the weight loss in addition to the expression of the IFNγ alone.
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)*	The mice have been provided with complan, diet gel and seeds. Body weight and mouse condition will continue to be monitored regularly. Information gained from this study will provide valuable information to feed into future study design. This may include submission of an amendment to allow for greater weight loss where there are no accompanying clinical signs, as a lower vector dose is likely to be insufficient for the disease model.
7. Reported by (name)	Rachel Watson
8. Date	19/02/2024

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Inspector’s comments and recommendations:

Comments and recommendations	

Inspector name	
Date	

Home Office

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	30/09/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses wildtype animals to gain control data for the disease model using varying concentrations of plasmid to induce expression of a pro-inflammatory agent. This study forms part of a wider programme investigating human *APOL1*-associated kidney disease. 30 mice (15 males and 15 females) were included in this study. They were dosed with five different concentrations of vector ranging from 0.05 – 2 µg, or vehicle control. This vector induces overexpression of a pro-inflammatory agent (IFNγ) when administered by hydrodynamic injection. Mice were administered with the vector by hydrodynamic injection on Wednesday 27/09/23. Due to the failure of some injections, each dose was administered to 3-4 mice. Mice recovered well from the hydrodynamic injection procedure. Mice were provided with hydrogel and seeds. Mice were weighed on Thursday 28/09/23. Some small body weight loss was observed, as expected after anaesthesia and a hydrodynamic injection procedure. Mice were weighed again on Friday 29/09/23, where weight loss was found to have increased. All mice were well, displaying no clinical signs. Mice were provided with diet get and seeds. This morning (Saturday 30/09/23), mouse 19 (female) was found to be displaying clinical signs (hunched) and was displaying body weight loss of -19.8% from the day of injection. She was immediately culled. This mouse was from the top dose (2 µg) dose group. Upon weighing of the remaining mice, body weight loss of greater that 15% was also observed in another 6 mice, however these 6 mice all appeared well and were not displaying clinical signs. Two of these mice (mouse 1, -16.4% and mouse 13, -18.1%) were also from the top dose and were culled. (This is all animals who received the top dose.) However,

	we wish to request to keep alive the remaining mice from the lower dose groups. The humane end point within the PPL is that mice who display body weight loss of 15% sustained over 2 consecutive days will be killed. We have four mice who are currently displaying body weight loss of greater than 15% (-15.4%, -16.0%, -17.4 & -15.4%) but who appear well and are not displaying any clinical signs. The mice have been provided with diet gel and seeds. Mice are due to remain on study for a further 4 days (until Wednesday 04/10/23). **We ask that we be permitted to keep all remaining animals on this study who display greater than 15% body weight loss but do not display any accompanying clinical signs alive. Any mice that display greater than 20% weight loss or display clinical signs will be culled by a Schedule 1 method. The data gained from these mice will provide vital information to feed into future study design.**
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the time-sensitive nature of this request, the NACWO and NVS have been sent this report alongside submission.
5. Cause of Problem, if known	
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)*	The mice have been provided with diet gel and seeds. Body weight and mouse condition will continue to be monitored regularly. Information gained from this study will provide valuable information to feed into future study design. This may include adjustment of the vector dosage, or submission of an amendment to allow for greater weight loss if the lower doses of vector are found to be insufficient for the disease model.

7. Reported by (name)	Rachel Watson
8. Date	30/09/2023

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	07/11/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 20 mice on the study. Yesterday (07/11/23), 20 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration and volume. The IFNy vector was dosed at two concentrations, at two different volumes, giving a total of 5 experimental groups. For each group, 2 males and 2 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received hourly clinical evaluation and scoring. Mouse 11 was found dead prior to the 1-hour clinical scoring.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the normal recovery of the remaining 19 mice, advice was not taken from the NACWO or NVS at the time. Tissues were immediately taken from the mouse upon death and our histologist will process the tissues to try to ascertain cause of death.

5. Cause of Problem, if known	The mouse was not in a rigor mortis state when found, and due to the regular checks, this death was likely very sudden. Mouse 11 received the lower dose volume and control vector, therefore the death was unlikely to be due to the substance injected. More likely, which will hopefully confirmed by histopathology, it was due to the hydrodynamic injection itself. We expect to lose a small proportion of animals at the point of injection while using anaesthesia but have yet to have a mouse that died shortly afterwards.
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)*	All other mice continue to be closely monitored, and no other adverse effects have been seen. We will consult with the NACWO and NVS at the first sign of any other issues and take steps to mitigate risk and evaluate the procedure if it happens again.
7. Reported by (name)	Amy Warner
8. Date	08/11/2023

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Inspector's comments and recommendations:

Comments and recommendations	Thank you for submitting this PPL SC18 Notification. In making this report, you are compliant with PPL Stand-ard Condition 18. The content of this SC18 report has been read and the mat-ter can now be considered CLOSED. Please continue to inform us of any other similar inci-dents should they occur.
Inspector name	Karen Mason

Date	09/01/2024

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	09/11/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice do not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 20 mice on the study. On 07/11/23, 20 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration and volume. The IFNy vector was dosed at two concentrations, at two different dose volumes, giving a total of 5 experimental groups. For each group, 2 males and 2 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received hourly clinical evaluation and scoring. Mouse 11 was found dead prior to the 1-hour clinical scoring (detailed in a SC18 sent 08/11/23). Today a mouse was found dead (mouse 15). There is nothing detailed in the records to indicate any significant clinical signs prior to this, and bodyweight was stable (-1.6% BWL from day 0 yesterday). The researcher who weighed the mice yesterday did mention that the mouse looked to be slightly less lively than his cage mates, but we did not feel this raised concern for action given the lack of clinical signs or body weight loss, the spontaneous movement around the cage, and normal response to stimuli.

4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NVS and NACWO were informed upon finding the animal, and we asked that the tissues are sent for clin path analysis.
5. Cause of Problem, if known	We are unsure of the cause of death for this animal, but upon weighing the rest of the cohort we found a number of other animals showing clinical signs and/or weight loss exceeding the humane endpoint. These mice have been sch1 killed, and two that exceeded the BWL humane endpoint have been reported in a separate SC18 report. We therefore believe in this case that the dose and/or dose volume of IFN gamma may be too high. Previous pilot studies where lower doses and volumes were used did not produce sufficient IFN gamma expression, so we have not as yet found the correct dose and volume for the disease model.
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)*	Remaining mice continue to be closely monitored. Any tissues sent for analysis will inform on possible cause of death. Lower doses and/or dose volumes will be chosen for further pilot studies.
7. Reported by (name)	Amy Warner
8. Date	09/11/2023

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Inspector’s comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	09/11/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice do not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 20 mice on the study. On 07/11/23, 20 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration and volume. The IFNy vector was dosed at two concentrations, at two different volumes, giving a total of 5 experimental groups. For each group, 2 males and 2 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received hourly clinical evaluation and scoring. Mouse 11 was found dead prior to the 1-hour clinical scoring (detailed in a SC18 sent 08/11/23). Today several mice (mouse 3, 7, 8, 9, 13, 17, 18, 19) were found to have clinical signs and were sch1 killed as per the humane endpoint. Two of the mice exceeded the humane endpoint for body weight loss (mouse 3, -16.9% from -6.2% yesterday, mouse 9, -18.9% from -5.5% yesterday). Mouse 9 was noted yesterday to be slightly pilo but not of concern enough to warrant action.

4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NVS and NACWO have been notified and we will keep them informed on the condition of the remaining animals, including discussion while observing the animals if they wish.
5. Cause of Problem, if known	As mentioned in part 1 of today's SC18 reports, we believe the dose and/or volume of IFN gamma may be too high. Previous pilot studies where lower doses and dose volumes were used did not produce sufficient IFN gamma expression, so we have not as yet found the correct dose and volume for the disease model.
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)*	Remaining mice continue to be closely monitored. Any post-mortem serum levels will inform on how to continue with lower doses and/or dose volumes in pilot studies.
7. Reported by (name)	Amy Warner
8. Date	09/11/2023

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	10/11/2023
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice do not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a newly imported line of mice expressing the G1 variant of *APOL1*. There are 20 mice on the study. On 07/11/23, 20 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration and volume. The IFNy vector was dosed at two concentrations, at two different volumes, giving a total of 5 experimental groups. For each group, 2 males and 2 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received hourly clinical evaluation and scoring. Mouse 11 was found dead prior to the 1-hour clinical scoring (detailed in a SC18 sent 08/11/23). Yesterday (08/11/23) several mice were found to have clinical signs and were sch1 killed as per the humane endpoint. Two of the mice exceeded the humane endpoint for body weight loss (detailed in a SC18 sent 09/11/23). Today, two additional mice (mice 12 and 20) exceeded the humane endpoint for body weight loss. The progression of weight loss (8th, 9th & 10th Nov) for these mice was:

	Mouse 12: -7.3%, -11.7%, -19.4% Mouse 20: -3.5%, -9.7%, -17.6% Mouse 20 was also displaying intermittent hunching and piloerection.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	A discussion was had with the NACWO regarding this study this morning and he and the NVS will be kept informed of next steps. The aim is for the NACWO to observe the next round of injections.
5. Cause of Problem, if known	As mentioned in yesterday's SC18 reports, we believe the dose of IFN gamma vector may be too high. Previous pilot studies where lower doses and dose volumes were used did not produce sufficient IFN gamma expression, so we have not as yet found the correct dose and volume for the disease model.
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)*	All mice from this study have now been sch1, as the study terminated today. Post-mortem serum levels from those sch1 yesterday are being determined today and will inform on how to continue with lower doses in future pilot studies. We have also collected tissues from the mice with clinical signs/weight loss to further investigate the reasons for the adverse reactions. We have also analysed the data for the different dose volumes tested and will be using a dose volume at the lower end of the range for hydrodynamic injections for the next pilot study. We plan to use Complan (high nutrient supplement) to assist with weight loss in future studies. We will also add additional afternoon checks for the first 48 hours of the next pilot study (or longer if required).
7. Reported by (name)	Rachel Watson
8. Date	10/11/2023

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Inspector's comments and recommendations:

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Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	23/01/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a line of mice expressing the G1 variant of *APOL1*. There are 22 mice on the study. On 23/01/2024, 10 wild type and 12 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration. The IFNy vector was dosed at two concentrations, giving a total of 3 experimental groups. For the control sequence group, 3 males and 3 females were injected, and for the IFNy groups 4 males and 4 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received at minimum hourly clinical evaluation and scoring. Mouse 16 (*APOL1* G1 heterozygous, high dose IFNy vector group) was found dead at the 1-hour post dose check. Mouse 22 (*APOL1* G1 heterozygous, high dose IFNy vector group) was found with blood around the mouth, subdued and unsteady on its feet approximately 40 minutes post dose, it was immediately culled.

4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	NVS was present during dosing and for initial recovery from the procedure but was not present when the mice were found dead/culled. The NVS was happy with the procedures. Tissues were taken from both mice and provided to the NVS. These will be processed to try to ascertain cause of death.
5. Cause of Problem, if known	Mouse 16 was not in a rigor mortis state when found, and due to the regular checks, this death was likely very sudden. Upon post-mortem examination of Mouse 16 it was noted that there was blood pooled in the abdominal cavity which could be suggestive of a vessel being ruptured during the hydrodynamic injection. Mouse 22 was observed to have been active at approximately 35 minutes post dose, so its clinical condition deteriorated very quickly until it was observed again 40 minutes post dose and culled. Post-mortem examination of Mouse 22 also revealed signs of a possible vessel rupture with blood found in the stomach and lungs. Examination of the tissues will hopefully provide further insight into the cause.
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)*	All other mice continue to be monitored daily. No other adverse effects have been observed. We will consult with the NACWO and NVS at the first sign of any other issues and take steps to mitigate risk. For the next few studies we will assign a researcher exclusively for monitoring the animals in the first hour after injection, to fully characterise any further incidences of this adverse effect and aim to reduce any suffering.
7. Reported by (name)	Amy Warner

8. Date	25/01/2024

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Inspector's comments and recommendations:

Comments and recommendations	Thank you for submitting this PPL SC18 Notification. Further information is required as described below: • • training and competency records of the PILh(s) who performed the hydrodynamic injections. **Please note** that any additional information should be returned in the form by responding to this email and adding **the report ID 240132** to the email subject to assist with onward processing. **Update –** Thank you for providing the requested records, from which it can be seen that the PIL was assessed as competent and able to train others in September 2023. The deaths are likely to be procedurally related and due to blood loss. However, the NVS was content with the procedures at the time. Nevertheless, for future procedures of this type, you have instigated closer monitoring for the immediate post-surgical hour, which is sensible. Please do inform us of any other related incidents should they occur. The content of this SC18 report has been read by an ASRU Inspector and this matter is now CLOSED.
Inspector name	Giles Paiba
Date	28 February 2024 Updated 2 May 2024

Home Office

PPL Standard Condition 18 Notification Form

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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	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	27/01/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). The aim of this study is to validate the disease model in a line of mice expressing the G1 variant of *APOL1*. There are 22 mice on the study. On 23/01/2024, 10 wild type and 12 heterozygous mice were administered with vectors for the overexpression of a pro-inflammatory agent (IFNγ) by hydrodynamic injection. Two different vectors were used, one containing control sequence and one containing the coding sequence for mouse IFNγ. The control sequence vector was administered at a single dose concentration. The IFNy vector was dosed at two concentrations, giving a total of 3 experimental groups. For the control sequence group, 3 males and 3 females were injected, and for the IFNy groups 4 males and 4 females were injected. The mice were placed under isoflurane anaesthesia for the hydrodynamic injection. Following dosing, mice were monitored until conscious and then received at minimum hourly clinical evaluation and scoring. Once recovered, mice were weighed daily and provided with supportive care including Complan. On 26/01/24 several mice showed signs of very slight dehydration, possibly due to the nozzles drying up as the mice recovered. The mice were provided with dishes of water for immediate rehydration and moved from the automated water system to water bottles. On 27/03/24, 4 days post-injection, mouse 19 (top dose IFNy vector) was found to have weight loss of -16.5%. This mouse had a slight hunch and was slightly less

	active than his cage mates. The mouse was killed by sch1.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Due to the incident occurring at the weekend advice was not taken at the time, however, the member of unit staff who was working at the time was made aware.
5. Cause of Problem, if known	Mouse 19 had not displayed any clinical signs prior to this day, with a weight loss trajectory or -5.1%, -10.3%, -8.2% for the three days prior to the day it was removed from study. As there were previous issues of dehydration within the study, this may have been a contributing factor. However, it is also possible that the expression of IFNy induced by the vector also contributed to the weight loss, along with the effects of hydrodynamic injection itself. Serum and tissue samples were collected post-sch1 and will be analysed for IFNy levels to provide some insight into whether this mouse was expressing particularly high levels of IFNy.
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)*	All other mice continue to be monitored daily and remain well. For any future studies, mice will be moved to water bottles prior to injection to provide easier access to water, particularly during the initial post-anaesthesia & injection period.
7. Reported by (name)	Amy Warner
8. Date	30/01/2024

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	04/03/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). This study involves administration of vectors for the overexpression of IFNγ by hydrodynamic tail vein injection (HTVI). Several studies have been performed using varying doses of vector (0.05 – 5 ug) to validate the disease model in heterozygous mice. This study was designed to validate the length of IFNγ expression after injection of the vector and the subsequent increase in APOL1 expression and disease phenotype in the kidney. 17 mice (5 males and 12 females) were included in this study. They were dosed with 0.2 or 0.4 ug of either a vector encoding IFNγ (n=13) or the empty vector (n=4) on 13th February. Six mice were killed during the first 24 hours due to reaching the humane end point as they did not recover well from the HTVI procedure, which was within expectations for this procedure. The dose volume has been adjusted slightly for the next cohort of animals which has reduced the number of deaths in the first 24 hours. Today (4th March, study day 20), one mouse has been observed to have a 'hunch', which persists while the mouse is moving around the cage. This mouse remains alert and active, with no other clinical signs. It is felt that this 'hunch' is not indicative of illness. However, as hunched posture is a humane end point for this protocol, the mouse has reached this endpoint. This mouse was dosed with 0.4 ug of the empty vector and has remained well since then (almost three weeks). This study is due to finish tomorrow (5th March). We wish to request to keep this mouse alive for up to 20 hours to

	allow all terminal samples to be collected. Collection of the samples a day ahead of schedule could affect the power of the study, therefore, to avoid losing the data from this mouse we would require keeping him alive until the planned terminal day. The data gained from this study will provide vital information regarding the disease model and will feed into future study design, particularly into determining the appropriate length for future studies.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NVS has observed the mouse and is happy to support our request to keep alive. She observed the mouse to be active in the cage and bright, with the hunch potentially an anatomical/skeletal hunch rather than a sign of illness.
5. Cause of Problem, if known	The cause of the problem is unclear at this time.
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)*	The mouse has been observed by a member of our team and the NVS and is active and bright, with no immediate action thought to be required. Upon killing tomorrow, a post-mortem will be performed to determine if there are any obvious abnormalities to explain the hunched posture.
7. Reported by (name)	Rachel Watson
8. Date	04/03/2024

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Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	

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Establishment Licence name	University of Cambridge (Mira)
Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	19/02/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). This study involves administration of vectors for the overexpression of IFNγ by hydrodynamic tail vein injection (HTVI). Several studies have been performed using varying doses of vector (0.05 – 5 ug) to validate the disease model in heterozygous mice. A dose of 0.2 ug vector was determined to be most suitable for future studies, however, it was determined that studies needed to move into homozygous mice to generate the required disease phenotype. 22 mice (7 males and 15 females) were included in this study. They were dosed with 0.2 ug of either a vector encoding IFNγ (n=17) or the empty vector (n=5) on Friday 16th February. One mouse died immediately at the time of HTVI and three mice were killed during the first 24 hours due to reaching the humane end point as they did not recover well from the HTVI procedure, which was within expectations for this procedure. Today (Monday 19th February, study day 3), three mice are exhibiting weight loss of greater than 15%. One of these mice was also exhibiting mild hunching and has been killed according to the humane endpoint. The remaining two mice (mice 3 & 8) are both well, displaying no clinical signs. The humane end point within the PPL is that mice who display body weight loss of 15% sustained over 2 consecutive days will be killed. The weight loss for these mice since HTVI is as follows: Mouse 3: -6.3%, -14.8%, -16.5% Mouse 8: -5.6%, -13.8%, -15.3%

	In both cases the rate of weight loss has slowed, and we have observed a stabilisation and recovery of weight loss in several other mice on study from study day 2 to study day 3. This matches the pattern observed in the previous study in heterozygous mice where weight loss stabilised from study day 2 to 3 and mice began to then regain some of the weight lost. The mice have been provided with supplements (seeds, complan) throughout the study and these are being refreshed regularly. We wish to request to keep alive these mice up to a maximum of 20% weight loss without associated clinical signs. The mice are due to remain on study for a further 7 days (until Monday 26 February), with dosing of therapeutics commencing on Wednesday 21st February. The data gained from these mice will provide vital information regarding the disease model in homozygous mice and the potential efficacy of a novel therapeutic and will feed into future study design.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO and NVS have observed the mice and are happy with our proposal to keep mice 3 & 8 alive.

5. Cause of Problem, if known	The weight loss is due to a combination of the expression of IFNγ and likely the subsequent increase in APOL1 expression and renal phenotype in the mice. Mice in this study dosed with empty vector are displaying much lower levels of weight loss, with some of this group now recovered back to starting body weight from the HTVI. The weight loss observed in these mice is greater than that observed in heterozygous mice, indicating that the renal phenotype caused by increased APOL1 expression is playing a part in the weight loss in addition to the expression of the IFNγ alone.
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)*	The mice have been provided with complan, diet gel and seeds. Body weight and mouse condition will continue to be monitored regularly. Information gained from this study will provide valuable information to feed into future study design. This may include submission of an amendment to allow for greater weight loss where there are no accompanying clinical signs, as a lower vector dose is likely to be insufficient for the disease model.
7. Reported by (name)	Rachel Watson
8. Date	19/02/2024

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Inspector’s comments and recommendations:

Comments and recommendations	

Inspector name	
Date	

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Establishment Licence number	X81BD37B1
Name of PPL holder	Amy Warner
PPL number	PP1533650
Protocol no	6
Protocol title	Murine models of inflammatory renal disease
Severity classification	Moderate
1. Date of incident	23/02/2024
2. Species	Mouse

3. Brief details of study and how the limits or constraints have been breached	The aim of this programme is to test novel therapeutic agents in a model of renal disease. The study uses transgenic mice expressing the G1 variant of the *APOL1* gene, used to mimic human *APOL1*-associated kidney disease. Data from similar transgenic mouse lines in the literature indicates that mice to not exhibit kidney disease until challenged with an inflammatory agent, such as the cytokine interferon gamma (IFNγ). This study involves administration of vectors for the overexpression of IFNγ by hydrodynamic tail vein injection (HTVI) on study day 0. Several studies have been performed using varying doses of vector (0.05 – 5 ug) to validate the disease model in heterozygous mice. A dose of 0.2 ug vector was determined to be most suitable for future studies, however, it was determined that studies needed to move into homozygous mice to generate the required disease phenotype. Dosing with novel therapeutic or vehicle control was started on study day 5. 22 mice (7 males and 15 females) were included in this study. They were dosed with 0.2 ug of either a vector encoding IFNγ (n=17) or the empty vector (n=5) on Friday 16th February. One mouse died immediately at the time of HTVI and three mice were killed during the first 24 hours due to reaching the humane end point as they did not recover well from the HTVI procedure, which was within expectations for this procedure. A further mouse was killed on study day 3 due to weight loss and mild hunching. These mice were across both treatment groups, with these adverse effects thought to be related to the hydrodynamic injection procedure. One further mouse was culled on study day 6 due to displaying subdued behaviour and therefore reaching the humane endpoint, this was a mouse that had received the control vector and vehicle dosing. There was a previous SC18 KAR request submitted for this study (Case ID 240299) which was granted for two mice who experienced weight loss >15% but were not

	exhibiting clinical signs. These mice have both gained weight and remain well. Today (study day 6, Friday 23rd February), mouse 14 was displaying body weight loss of -16.5%, therefore breaching the humane end point. The mouse was also displaying clinical signs (hunching, piloerection, subdued behaviour) and was immediately culled. The body weight loss the previous morning was -5.2%, and the mouse had been checked and dosed on three occasions in the previous 24 hours, with the mouse being in good condition and displaying no clinical signs at 10.30pm on Thursday evening when last dosed and checked. This indicates that the mouse experienced a rapid decline This mouse had received the vector encoding IFNγ and was receiving treatment with a novel compound. Other mice in this treatment group (n=4 still on study) remain well.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	The NACWO & NVS have both reviewed this SC18 and have provided input throughout this study.
5. Cause of Problem, if known	A post-mortem was performed which did not provide any obvious causes for weight loss and clinical signs. It may be due to a combination of the expression of IFNγ and subsequent increase in APOL1 expression and renal phenotype in the mice combined with the stress of 3x/day dosing, however the other mice in this treatment group remain well. Tissues have been collected, which once analysed may provide further insight into the cause of the decline. The water bottle on the cage was checked to ensure water was available to the mouse. It was in a cage of 4 with the other mice in the cage not exhibiting significant weight loss, which can exclude dehydration as a possible cause.

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)*	The mice have been provided with complan supplement throughout the study as supportive care. Body weight with continue to be recorded daily and mouse condition will be examined 3x/day at the time of dosing (8.30am, 2.30pm & 10.30pm). The study is due to finish on Monday 26th February (study day 10).
7. Reported by (name)	Rachel Watson
8. Date	23/02/2024

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Inspector's comments and recommendations:

Comments and recommendations	Thank you for submitting this notification and thereby complying with standard condition 18 of your project licence. The content of this SC18 report has been read by an ASRU Inspector and this matter is now CLOSED.
Inspector name	Giles Paiba
Date	28 February 2024