Retrospective Review of Project Licences

Project Licence Retrospective Review

"The overall purpose of retrospective review in the general sense of '*following the development and outcome of projects'* is, wherever possible, to reduce the harms and increase the benefits of every project at an establishment. The aim is to improve both animal welfare and the quality of science and to help inform future debate on these issues." [RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)]

At the University of Cambridge one (or possibly more) of the following will determine when your licence will be requested for review:

a. 12 to 6 months before your existing licence is due to expire if you have no plans to write a new licence;
b. 15 to 12 months before your existing licence is due to expire if you plan to write a new project licence;
c. 3 months before you terminate your licence if you intend to revoke your licence before its due expiry date;
d. When there is a change of project licence holder (in this case the person currently holding the licence should complete the retrospective review before the amendment is made to change the project licence holder);
e. As determined by the AWERB Standing Committee. The AWERB Standing Committee may decide a licence should be reviewed at a specified time or times based for example on the severity of the work, novelty of the models etc..

The information you provide in or attached to this document must reflect data generated only from work carried out under the licence under review. Please do NOT copy and paste text that appears in your current licence unless requested to do so.

You are not restricted by the size of the text boxes on this form; they are designed to expand to accommodate your text. However please remember that AWERB Standing Committee members, which will include your colleagues, have to review the information you provide so please be informative and concise and do not include information that is not requested. Please remember to use Plain English, where possible.

Please be aware that Retrospective Reviews, as with your current and previous project licences, can be requested under the Freedom of Information Act 2000. It is therefore wise to take care when completing this document and ensure that it does not contain typographical errors, personal details or any sensitive information pertaining to Intellectual Property.

A copy of the final agreed version of this document and any advice provided by the University AWERB in relation to this document will be forwarded to your local Home Office Inspector.

If you use abbreviations could you please ensure the first time the abbreviation is used that it is spelt out fully with the abbreviation in brackets.

Finally, if Home Office has identified your licence as one that required a Retrospective Assessment please still complete this template. The information gathered will be used by AWERB to undertake their required task to review your work and can be used to complete the Home Office Retrospective Assessment template. The latter can be achieved by copy and paste will a further update just before submission to include work you have complete between when you fill in this template and the expiry of your licence, which is when your Retrospective Assessment will need to be sent to the Home Office.

Thank you.

1. General Information:

Project licence holder to complete if information provided is incorrect

Name:	Professor Timothy Cox	
Contact details: Address & tel.	Prof T M Cox Department of Medicine, University of Cambridge, Box 157. Level 5 Addenbrooke's hospital, Hills Road, Cambridge CB2 0QQ	Lysosomal Disorders Unit, Box 135, Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ. United Kingdom. Tel: +44-(0)1223-274634 (clinic)
Project No.	P09AD3ABE	
Project title	Therapeutic development for sphingolipid diseases	
Project purpose	The overall aim of this application is to generate data necessary to expand understanding of the fundamental processes which result in sphingolipid disease and use this data in the development of effective treatments. This will be achieved by considering the following 4 overall objectives: (1) generation of data which will further elucidate the fundamental biological processes occurring in these diseases; (2) generate data in animals which verifies the efficacy and safety of gene transfer techniques in the treatment of GM2 gangliosidosis (Tay-Sachs and Sandhoff disease); (3) developing or repurposing inhibitors of sphingolipid formation (substrate reduction therapy) either to reduce damage to the brain (Krabbe disease) - or decrease the risk of cancer (in Gaucher disease and related conditions); (4) manipulating of murine genetics to determine the strong host factors that influence the appearance and behaviour of these diseases.	

Date licence was granted	12th December 2017
Date of retrospective review is sent to the PPLh	19/12/22

The following are the triggers for licence Retrospective Review:
If you are completing this form because you have been asked to complete a Retrospective Review and our UBS staff have completed this box for you. Please check that you agree with the selection made and correct if you believe the information is incorrect.

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

Note to licensees:
In the case of **option D** if the new project licence holder's name has not been filled in please add this information to this box.
In the case of **option E** please insert the name of the person from whom you took over this licence and the date when you became licence holder.

2. Animal Welfare/Recorded Harms:

2.1 Using the table below please provide the information requested.
How to complete this table: Return of procedures count animal numbers from 1st January to 31st December. In the table below if your licence was granted part way through the year count year one as the time from when the licence was granted to the 31st December of that year. Year two will be from January to December etc. until the final year for which the report is requested and count from 1st January to the requested report date. You can insert rows as necessary. The figures provided can be obtained from your Home Office returns.

Protocol No.	Species	Protocol severity category	Total est. number	Actual number used	Actual severity recorded					
					Non-recovery	Sub Threshold	Mild	Moderate	Severe	Totals
1	Mice	Mild	684	1st year			14			14
				2nd year			15			15
				3rd year			5	22		27
				4th year			63			63
				5th year			67			67
				Total			164	22		**186**
2	Mice	Moderate	50	1st year						
				2nd year						
				3rd year						
				4th year						
				5th year				2		2
				Total				2		**2**
3	Mice	Moderate	400	1st year						
				2nd year			63	16		79
				3rd year						
				4th year				11		11
				Total			63	27		**90**
4	Mice	Mild	3324	1st year						
				2nd year						
				3rd year			2			2
				4th year						
				Total			2			**2**
5	Mice	Mild	8666	1st year		173	69			242
				2nd year		416	82	1		499
				3rd year				18		18
				4th year		103	335	7		445
				5th year		125	288	2	9	424
				Total		817	774	28	9	**1628**
6	Mice	Moderate	13872	1st year	`	771			8	779
				2nd year		420	56	1	8	485
				3rd year		270	15	12	6	303
				4th year						
				Total		1461	71	13	22	**1567**
7	Mice	Moderate	1000	1st year			202	25		227
				2nd year			157	2		159
				3rd year		6	10	25		41
				4th year		6	10	25		41

7 (contd)	Mice	Moderate	1000	5th year		1	16	2		19
				Total		13	395	79		**487**
8	Mice	Moderate	4776	1st year		60	342	119		521
				2nd year		133	15	100		248
				3rd year	33	42	10	30		115
				4th year						
				Total	33	235	367	249		**884**
9	Mice	Moderate	400	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
10	Mice	Moderate	576	1st year		47	42	41		140
				2nd year						
				3rd year						
				4th year						
				Total		47	52	41		**140**
11	Mice	Moderate	300	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
12	Mice	Moderate	600	1st year						
				2nd year						
				3rd year				2		2
				4th year				24		24
				5th year		1	16	2		19
				Total		1	16	28		**45**

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

2.2 Does the information provided in the table reflect the projected incidence of harm provided in the adverse effects for each protocol?

YES, in general.

If not please indicate where this was not the case and whether the projected level of suffering was more or less than you anticipated when you first wrote the licence. If the severity category for a protocol was amended during the tenure of the licence please include this information in the reasons box.

(Not applicable)

Protocol number	Severity higher	Reason
5		9 mice found dead in litter after post-natal day 5. Attributed to unusual segregation of rare genes in the parental backgrounds that we had found were in single doses determinants of autoimmunity. We did not breed further from the parents and did not encounter the problem again.

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

Given the number of animals used, there were very few unexpected adverse effects (< 1%); this reflects the long duration of the programme of work and familiarity with the different models of disease. An occasional animal has been found dead but no *pathological* cause has been established in any such instance (see below).

The animals used represent continued use of those generated by Protocol 5 ('Breeding and maintenance of GA animals' – mild). For completeness, it should be made clear that the research requires experimental comparison with control GA mice of various Gaucher strains in protocol 7 ('Ageing and Phenotyping of Gaucher-related animal models' - moderate).

We have developed and studied mouse strains of nonneuronopathic Gaucher disease for 13 years. Induction of disease in genetically modified (flox/flox) mode requires daily repeated parenteral injection of an alpha-interferon analogue in neonatal mice.

As a result of careful breeding and interbreeding with other strains and close observation, we have have identified mice that have different manifestations of human Gaucher disease expressed with varying severity. We suspect autoimmune complications as a cause of liver disease and subsequent cancers in these animals.

A clear example of our experimental system in different strains of mice, includes those also lacking CD1 on their immune cells: this protein presents the Gaucher disease lipids to the Immune system and has been introduced during the lifetime of the work to work out if this will reduce the disease manifestations during ageing.

The final question is addressed in Protocol 12 explores whether the manifestations leading to cancer in the mice (as in Gaucher patients) can be prevented by dietary exposure to an approved treatment recently introduced in the clinic.

For completeness in this review all section 18 reports from 2018 onwards are set out:- three Project Licence Standard Condition 18 reports were submitted: (i) 02-FEB-2021; (ii) 22-APR-2022; (iii) 30-JUN-2022. All relate to Gaucher disease in Protocol 12, ('Testing interventions for Cancers' - moderate).

(i) A 14 day-old mouse was found dead while was in the parent cage after two days of the second dose of an interferon inducer (poly IC) injection via intraperitoneal route. The body was eaten with only the lower part of the animal remaining. The animal had received the intraperitoneal injections following the established and tested protocol to induce Gaucher disease phenotype. The mouse was weighed on the next day and monitored every day after the injection - no prior abnormalities were found. The other 10 animals of the litter appeared healthy - all had had the injections. Based on the genotypes, the two litters of 11 animals were born from the trio (male and two females) breeders on the same day and were indistinguishable. Neonatal deaths regardless the genotypes have been observed in this GD1 strain and could be related to unexperienced parents or large litters. The death during poly IC

injections has happened for the first time in this strain since the establishment of the colony in 2009. The death likely was caused by non-procedural harms to the offspring and parental behaviour. It has not recurred. Animals were active and did not show any clinical signs: no obvious abnormalities in remaining organs were found at post mortem.

(ii) Adult CD1-Gaucher mice on the medicated diet treatment under protocol 12 step 1 received substrate inhibitors orally in food ad libitum for 9 months since July 2021. In a cage of 4 animals, three mice had lost more than expected body weight:

(a) Mouse COAP14.5a reported to have lost '17.38 %' below of the highest achieved weight which the exceeded humane endpoint of 15% weight loss However, the mouse was re-weighed shortly after and the weight increased to 27g (gain 2g and restored weight. (b) Mouse COAP14.5h TRBR weighed in the morning 22 April: weight was 26g – which was 12% reduction of the highest achieved weight. The mouse was re-weighed again, the weight increased to 28.6g (+2g). (c) Mouse COAP 14.5e TR morning weight was 27.1g, the mouse was re-weighed in three hours later, the weight increased to 28.6g. Review of the data caused showed that the animals in effect maintained the same body weight with exceptions when significantly increased values were recorded that affected estimation of short-term weight trends
With advice from HM Inspector, a policy of carefully monitoring to prevent recurrence of this incident was put in place to ensure consistent weighing practices

(iii) In June 2022 one animal in a cage of two females (under 10% weight loss monitoring as established on the licence with the support of experienced technical staff was found to have lost -16.2% maximum achieved body weight. (limit -15%).
Crunched (drug-dosed) food was found in the cage but with an empty feeding receptacle. Both mice in the cage were active and healthy – no tumours or signs of liver disease were detected and condition of skin and fur was judged healthy. HM Inspector notified:-

1. in summary the food hopper appeared not to have been filled, resulting in a mouse exceeding the authorised 15% weight loss. However, there was food in the cage (these mice appear to be fussy eaters) and the weight was re-gained within 24 hours.
2. As this mouse had exceeded the maximum permitted weight loss it should have been culled unless authority to Keep Alive had been granted by ASRU. To do this the SC18 submission should state KEEP ALIVE REQUEST in the subject heading, and must be supported by the NVS and submitted immediately. These are then dealt with within hours of being received (office hours). NACWO and NVS had not informed the licence holder of this requirement.
3. These actions were not strictly followed but after discussion and internal review with all parties, a policy of vigilance and prompt reporting agreed.

   NB. There have been no recurrences or further cases: the animal in question remained healthy throughout and the experiment was completed with termination as required after the preordained period of drug exposure.

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

Protocol No.	% and number of animals killed or unexpectedly found dead
5 (intended continued use to 12)	< 1% (9 of 1628 year 5 was the maximum in any one year)

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

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

Protocol No.	If your numbers were less that estimated please explain why.
1-4	At the termination of this licence only six strains were considered essential for later research and saving sperm was suitable in several cases.
5,6,7,8, 9, 10 &12	By the time the licence had been initiated several experimental breeding strains were established and the need for expansion was less than might have proved to be the case. The pandemic, lock-down and slow-down, movement of facilities from West Forvie and CBS exclusively to AMB with the time taken to establish working on various floors; departure of staff active experimental staff SW due to retirement; KB and EZ due to termination of grants; AT due to technical incapacity and resistance to conduct of animal procedures; CH due to termination of her foreign PhD studentship and illness (protocol 11 was never initiated as a result) and insufficient funds to support animal work from 2020 (MBC-G) were the principal reasons for less use of animals – in particular, we had expected that if our gene therapy vectors for GM2 gangliosidosis were to be supported by commercialization for full clinical development, the biosafety and efficacy testing many hundreds more of GA mice would have been needed. Finally EP, active experimentally throughout the 5 years under the Act using strains of mice and was adequately funded for the conduct of genetic characterization and mapping - as well as directly translational therapeutic science - had minimized the requirements for animals. This was outcome of advanced observation and phenotyping and strain identification by

	molecular haplotyping: thus early in the project, genetically distinct characteristics resembling human Gaucher disease had emerged in sub-strains that could bred out selectively for study.

2.6 Have you re-used animals?

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

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

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

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

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

Circumstances and nature: Breeding protocols – very few animals were excess to requirement and unused
Estimated number or percentage of animals wasted and if appropriate provide this information by protocol: Protocol 5 – 3 in 2022, 2 mild, I moderate. <1%

2.8 For licences that authorise the breeding and maintenance of genetically altered animals: a) explain how you optimise breeding efficiency and
b) explain what use is made of any excess stock animals.

(a) see above in 2.7 – once the interbreeding had been conducted and the first of several recombination events had occurred around the murine Gba1 and Cd1d loci (as suspected in retrospect), the sub-strains of GA animals with different proclivities (eg. autoimmune hepatitis and liver cancer or paraproteins with B-cell proliferation/myeloma/lymphoma) were worked out, the background strain origins were traced by genotyping and haplotype analysis – thus favouring identification and allowing studies of pathogenesis by efficient propagation with minimal wastage. (b) excess stock animals were potentially made available to others in the Clinical School scheme operational on the campus, unused animals were terminated promptly when we took full stock of the experimental plans.

2.9 If you breed genetically altered animals are your strains recorded on a database that can be

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

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

The work is of considerable scientific and possibly commercial biotech/medical interest and we would welcome experimental engagement within the local academic environment to expedite our work. This is not yet possible because of intellectual property needed for ultimate development in the clinic in Gaucher disease.

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

We would welcome collaborations to expedite our work. As of now, long-range DNA sequencing is being conducted to refine the candidate chromosomal regions in which the putative genetic determinants lie. This is being carried out on tissue samples harvested from the experimental animals.

Any release of material before publication would jeopardise IP and its exploitation in the clinic especially since our finding immediately suggests different ways to manage Gaucher disease.

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

Please explain/provide examples.

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

Species	Source	Approx. percentage

2.12 During the tenure of the licence have you requested an amendment to increase/decrease animal numbers? **YES**

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

Protocol No.	Original estimated Number	Amended estimated number
4	250	3324
5	600	8666
6	1200	13872
7	250	1000
8	800	4776
10	400	576

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

We originally considered (a) that we would have the staff, access (this was pre-pandemic) and funding to expand the research as described and (b) greater numbers would be needed to fulfil the aims realistically. The estimates are very difficult and an original estimate did confuse the annual with the total Numbers.

In the event, far fewer animals were used because: (a) funding did not turn out as hoped, especially for mass testing of gene therapy vectors for human use in years 1-3 and (b) in large part a much more efficient reduction came about [2.5 above] through judicious breeding based on strain haplotype analysis and clear phenotypic segregation. Just before the licence was granted, after 5 years, we had obtained viable and unique breeders with a very complex genotype (Cre+/ GBA flox-flox/Cd1d1$^{-/-}$ /Cd1d2$^{-/-}$) which greatly accelerated the research in Gaucher disease strains.

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

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

Protocol No.	Original severity category	Amended severity category

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

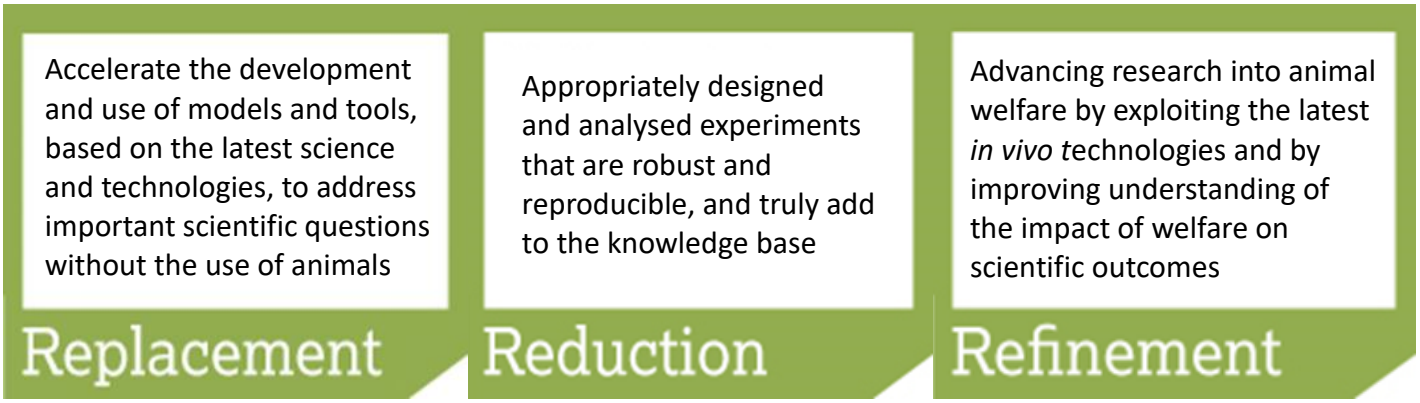
N/a

3. **3Rs:**

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

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

Definitions



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

Please explain.

At the time of application, no efforts were spared to seek alternative means to avoid animal research. This set of translational projects on these diseases with therapeutic aims directly relevant to human diseases present lack the technology for in vitro replacement or replacement by animals of less sentience (we have however made one recent foray into a scientific collaboration with a colleague who conducts experimental work in fish. Gaucher disease has been introduced into Zebrafish but this is pathogen research that is more suited to study in young Danio embryos).

3.2 Have you been able to reduce your animal usage? **YES**

If so, how have you achieved this (e.g. through experimental design, changes to the regulated procedures used)?

Experimental design was minimized at the time of licence submission. Reduced by (1) as a result of external factors as detailed (funding, access, staffing matters, time expiry on grants) and by technical action (2) in Gaucher research by highly targeted breeding programme based on phenotyping sub-strains (antibody testing for emerging autoimmunity) and haplotyping for the three main background strains each with different genetic predisposition to various disease manifestations observed in human patients.

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

Please explain:

Yes – we have participated in the Clinical School sharing scheme at the campus including affiliated institutes.

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

Mice only with complex husbandry using breeding protocols for interstrain crosses

Neurological: GA models of GM2 gangliosidosis and two models of Krabbe disease (twticher 2 J and twitcher 5 J); interbreeding with RIP kinase mutants (RIPK3 knockout and non-kinase RIPK1).

Gaucher disease: inducible type 1 non-neuronopathic strain; Cre strain; classical black 6 and R129 backgrounds; Cd1d1d2 knockout strain.

Blood sampling, genotyping (ear tag); perfuse fixation (non-recovery); administration of putative therapeutic sphingolipid biosynthesis inhibitors

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

Despite extreme challenges as listed we have been highly successful in the Gaucher disease work and have made progress in the study of the neuronopathic sphingolipid diseases (that affect the brain) both from the basic understanding of the disease but in both instances potentially therapeutic discovery for human application (translational research).

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

Securing adequate funds and commercial investment expected for gene therapy for the very rare Tay-sachs and Sandhoff diseases (despite £500,000 commitment from MRC Life Arc and £500,000 commitment from the University of Cambridge (Cambridge Enterprise); impossibility of judging animal expenditure accurately with very high costs; moving facilities; lack of competent (trainable) staff through retirement and funding challenges.
There is no true power of mitigation of such forces – more research per person was done by a fewer dedicated colleagues and it tends to be high quality but the economy of scale is impossible to recreate by other means.

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

YES

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

Breeding GA sub-strains of mice with Gaucher disease using haplotype analysis and developing dependable means to detect autoimmunity for accurate phenotyping of strains of interest.

To be published but this has been a 12 year+ project and many additional months of analytical work are needed before it can be submitted.

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

If not, why?

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

No refinement needed but we found the systematic foundation of the Body Condition Scoring system 1-5 to identify and monitor animals with precancerous disease and those with frank tumours useful.

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

Please explain.

The the 15% loss of maximum achieved body weight appeared to be mangeable and appropriate in most cicumstances and were not changed after two section 18 submissions and explicit requests for advice from HM Inspector whose guidance was very helpful.
We suspect that technical staff can encounter difficulties when there is hand over at weekends and when very pressed – if different balances are used we believe that inconsistent measurements can arise spuriously. In the event no experimental animals were inappropriately killed.

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

Perfuse-fixation (This was used in the Krabbe and Sanodhoff neurological mouse strains - 4% paraformaldehyde)

Were these killing methods the most refined? **YES**
Were they the most appropriate for your experimental data? **YES**

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

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

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

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

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

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

4. Scientific Progress and Benefit Achievements:

4.1 Taking into consideration that your licence may not be due to expire, has your research progressed as you expected? **Not completely**

4.2 When your licence is due to expire will you have completed the programme of work described in this licence? **We did not completely - but practically all the Gaucher work and some therapeutic as well as basic mechanistic discoveries in Krabbe disease have been realised satisfactorily - although the publication of the former will require continued analysis. Several manuscripts are planned for submission in 2023.**

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

1. ***Does the "host environment" of sphingolipids in which cells arise, drive the development of cancerous lymphoid tumours and myelomatosis and can the interactions between cells and their host be manipulated therapeutically?***

Research conducted during the tenure of this licence has clarified and advanced knowledge definitively on these points: lymphoid tumours, lymphoproliferation, autoimmune hepatitis progressing in some cases to liver carcinoma in Gaucher disease mice, has been found to be absolutely dependent on the mouse developing Gaucher disease with pathologically elevated sphingolipids but do not arise in any appropriate matched control population. The autoimmunity and liver disease appear strongly also to mirror severe hepatic disease and rare but ill-understood liver cancer in patients: this is a new experimental phenomenon that arose on interbreeding our congenic founder colony (that has inducible Gaucher disease) with another strain.

The two main manifestations (haematopoietic/lymphoid proliferation complicated by myeloma, and severe auto immune liver disease) segregated clearly with strains as demonstrated by haplotype analysis.

3. ***Can we find useful therapeutic effects of drugs (substrate-reducing drugs - SRT) which are intended to reduce the over-production of toxic sphingolipids that is the causal basis of this group of diseases?***

(a) The proliferative states and cancers are prevented in the mice by administration of eliglustat, as previously found for the lymphoma/myeloma during the course of work under the previous PPL- and confirmed here. This is an approved drug that serves as a specific inhibitor of glucosylceramide biosynthesis and at the doses given, corrects the overproduction of Gaucher disease-related sphingolipids. Of note, this agent is used worldwide as a first-line orally active agent for patients with type 1 Gaucher disease.

(b) During the course of this licence, we successfully explored the use of a novel investigational agent that inhibits UGT8, the enzyme, UDP-galactosylceramide transferase, which catalyses the first committed step in the formation of galacto-series sphingolipids whose recycling is defective in Krabbe disease and metachromatic leukodystrophy.

These neuronopathic sphingolipidoses are cased by an entirely different glycosphingolipid class present selectively in the nervous system and myelin. Our preclinical evaluation of an innovative biosynthesis inhibitor for the treatment of patients with Krabbe disease (and by inference the related disease, metachromatic leukodystrophy) was considered for substrate reduction therapy. This was

conducted as part of a collaborative research programme with Sanofi: there are indications now that this approach will be advance to the clinic for these dire diseases.

As shown in the attached publications arising from this work, we discovered early proof-of-principle in experimental Krabbe disease (twitcher strain mice) using a long-awaited inhibitor of the defining reaction for biosynthesis of galactosphingolipids: UGT8 catalyses the last step common to the de novo and salvage pathways from ceramides and is rate-limiting for formation of galacto-series sphingolipids that are the principal myelin lipids abundant in the nervous system. Since galactosylceramides are also the immediate source of neurotoxic psychosine when GALC is deficient, and the inhibitor markedly decreased this metabolite in tissues in twitcher mice (a disease model in a living vertebrate with myelinated nerve fibres), the case for targeting UGT8 is self-evident for therapeutic exploration in Krabbe disease and related metabolic diseases of the nervous system is consolidated by our work. Our research conducted under this licence is reported in a first paper:-

[Zaccariotto, E., Cachón-González, M. B., Wang, B., Lim, S., Hirth, B., Park, H., Fezoui, M., Sardi, S. P., Mason, P., Barker, R. H., Jr, & Cox, T. M. (2022). A novel brain-penetrant oral UGT8 inhibitor decreases in vivo galactosphingolipid biosynthesis in murine Krabbe disease. Biomedicine and Pharmacotherapy 149, 112808].

In this paper we showed that exposure to the inhibitor significantly decreased several characteristic inflammatory response markers without apparent toxicity to myelin-producing cells in wild type and mutant mice. Administration of the inhibitor before conception and during several breeding cycles to mice did not impair fertility and gave rise to healthy offspring - suggesting that there will be considerable margins for safe clinical development in humans.

4. ***Can we identify host factors that will modify the onset and severity of the sphingolipid diseases?***

(a) Gaucher disease (cancers and autoimmunity)

Identification host factors

This work, built up since 2009, has emerged as the most successful aspect of the translation research conducted during the tenure of this PLL.

Several manuscripts are in preparation (Pavlova etc al) and, subject to institutional Intellectual Property review and protection for human application, will be submitted for publication in 2023. The research is innovative and represents the outcome studies that enabled two recombinant events in the mouse genome that led to the identification and subsequent mapping of strong strain-dependent determinants of liver-focussed autoimmunity or B-cell proliferation in mice engineered with inducible deficiency of lysosomal glucoceramidase (Gba1) in haematopoietic stem cells. The candidate genes co-localize to a highly gene-rich cluster of immune-related sequences that are part of an evolutionarily conserved syntenic segment that harbours the human GBA1 gene and murine Gba on chromosomes 1q21.2 and 3, respectively.

We believe these studies to be of great importance in the general field of autoimmunity and its genetic determinants mediated by T-cell immunity and breakdown of tolerance to autologous glycosphingolipid antigens that are disturbed and anomalously displayed as a consequence of the genetic defect in Gaucher disease.

(b) GM2 gangliosidosis and Krabbe disease (sphingolipid diseases that cause severe persistent neurodegeneration)

Host Reaction

Krabbe disease is due to mutations in the *GALC* gene that encodes the lysosomal acid hydrolase, galactosylceramidase - this recycles sphingolipids that are abundant in nervous tissue and are associated with myelin. Homozygous deficiency of this enzyme is associated with loss of oligodendroglia and Schwann cells - cells critical for myelin integrity. In Krabbe disease, reduced metabolic turnover of galactosphingolipids causes dysmyelinaton accompanied by neuronal loss and inflammatory changes. Related to the therapeutic research in twitcher models above, under this licence we undertook a detailed study of twitcher strains, fully to understand the nature of cell death and the associated neuroinflammatory responses that may themselves show salutary responses to therapeutic intervention with RIPkinase and other implicated inhibitors of trhe offered targets.

(i) Cachón-González, M. B., Wang, S., & Cox, T. M. (2021). Expression of Ripk1 and DAM genes correlates with severity and progression of Krabbe disease. Human molecular genetics, 30(22), 2082–2099.

(ii) Cachón-González, M. B., Zhao, C., Franklin, R. J., & Cox, T. M. (2022). Upregulation of non-canonical and canonical inflammasome genes associates with pathological features in Krabbe disease and related disorders. Human molecular genetics, ddac299. Advance online publication.

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

2. ***Are the chosen gene therapy vectors to be used in human GM2 gangliosidosis trials (Tay- Sachs and Sandhoff diseases) safe and effective? If toxicity is seen, can it be ameliorated by medical treatments?***

With the failure at the last minute of venture capital fully to support our clinical development programme for gene therapy in GM2 gangliosidosis, including bioequivalence, safety and reiterated efficacy studies in the hands of third parties, we remain actively engaged with the rich translational environment for introducing our technologies of targeted gene therapy based secretion-recapture for lysosomal enzyme delivery to the CNS via local facilities.

The attached publication sets out what has been carried out in this field.

[Cachon-Gonzalez, M. B., Zaccariotto, E., & Cox, T. M. (2018). Genetics and Therapies for GM2 Gangliosidosis. *Current gene therapy*, *18*(2), 68–89]

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

Confirmation of the anticancer effects of the substrate-reducing treatment option for cancers complicating Gaucher disease and explore potential contribution of sphingolipid metabolites to other cancers such as lymphoma or myeloma occurring in the general context that are available models.

Finally defining the exact molecular causation of these tumours will be of deep significance for a large human burden in which a role for sphingolipid abnormalities has been suggested, but is unproven.

Identifying genes that influence neurological manifestations in Krabbe disease to improve therapy (based, for example, on substrate reduction combined with gene transfer, or on new drugs that act on the products of the modifier gene variants to affect the disease process).

The first preclinical evaluation of an innovative biosynthesis inhibitor for the treatment of patients with Krabbe disease and metachromatic leukodystrophy by substrate reduction therapy. This is part of a collaborative research programme and is expected to advance rapidly to the clinic.

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

Despite much local support, support from the MRC and international patient societies and advocacy groups - as well as a 'full house' of regulatory approvals in Europe and the United States, we have not have not succeeded in advancing our 20 year prior work on gene therapy into the clinic for Tay-Sachs and Sandhoff trials. Single patients treated with what we believe are inferior technologies have failed However, for the late-onset forms of these diseases, Cambridge, (PI Cox) is currently conducting phase 2 clinical trials as part of a pharmaceutical initiative in neurological Gaucher disease and late-onset GM gangliosidosis with a brain-penetrant substrate reducing, sphingolipid biosynthesis inhibitor.

4.7 Were you able to identify/develop/implement any new scientific or technical developments during the tenure of this licence? **YES**

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

We have used (i) classical genetics combined (ii) with two fortuitous recombination events to map a tight chromosomal region close to the Gaucher locus that encodes the enzyme, glucosylceramidase that is deficient in the disease. This region is shared by mice and humans: it contains genes, variants of which we have shown predispose to the cancers related to autoimmunity. Their effects are potentiated by the presence of the increased and different glycosphingolipids in the cellular environment. We have strong candidate genes but are currently seeking definitively to identify which of them drives this cancer and autoimmune process by (iii) nanopore sequencing. We contend that this work has more general significance across medicine and biology – genetic drivers will strong effects like this have not been identified before.

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

Please highlight in bold the publications which contain and meetings at which 3Rs achievements were disseminated.

Please only include publications arising from research undertaken under the authority of this licence. If you have papers in press please include these.

Publications in scientific journals:

Cachón-González, M. B., Zhao, C., Franklin, R. J., & Cox, T. M. (2022). Upregulation of non-canonical and canonical inflammasome genes associates with pathological features in Krabbe disease and related disorders. *Human molecular genetics*, ddac299. Advance online publication.

Zaccariotto, E., Cachón-González, M. B., Wang, B., Lim, S., Hirth, B., Park, H., Fezoui, M., Sardi, S. P., Mason, P., Barker, R. H., Jr, & Cox, T. M. (2022). A novel brain-penetrant oral UGT8 inhibitor decreases in vivo galactosphingolipid biosynthesis in murine Krabbe disease. *Biomedicine & pharmacotherapy 149*, 112808.

Cachón-González, M. B., Wang, S., & Cox, T. M. (2021). Expression of Ripk1 and DAM genes correlates with severity and progression of Krabbe disease. *Human molecular genetics*, *30*(22), 2082–2099.

Lecommandeur, E., Cachón-González, M. B., Boddie, S., McNally, B. D., Nicholls, A. W., Cox, T. M., & Griffin, J. L. (2020). Decrease in Myelin-Associated Lipids Precedes Neuronal Loss and Glial Activation in the CNS of the Sandhoff Mouse as Determined by Metabolomics. Metabolites, 11(1), 18.

Cachon-Gonzalez, M. B., Zaccariotto, E., & Cox, T. M. (2018). Genetics and Therapies for GM2 Gangliosidosis. *Current gene therapy*, *18*(2), 68–89.

Pavlova E.V., Archer J., Shatunov A., Pewzner-Jung Y., Futerman A.H., Cox T.M (March 2022) Sphingolipids regulate T cell self-tolerance and adaptive immune response.
Abstract only pending full manuscripts at Gordon Research Conference, Il Chiocco, Italy March 25-30 2022

Presentations at scientific meetings: examples
British Society for Cell and Gene Therapy
Gordon Research Conference in Sphingolipids
Oxford Rare Disease conference

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

4.9 Have you engaged with the general public by giving interviews about your work on radio, television, at a public meeting or debate or in a newspaper article? **YES**

Multiple patients meeting (Tay-Sachs and Sandhoff disease, Krabbe disease as well as Gaucher disease-related meetings as well as Cambridge Rare diseases Network) and also in conjunction with the Policy & Insights, Economist Impact, The Economist Group discussing the impact of rare paediatric neurological diseases (23-Feb-2022) and currently in Rare Revolution Magazine (ongoing 2022-23).

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

Interview, recording and correcting proof for publication eg 23-Feb-2022

5. Project Licence management information.

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

08-AUG-2018 (request submitted 20-APR-2018)

Category I (involving founders and/or breeding and maintenance)

(a) This will involve the **mild** procedures in **Protocol 1** (Superovulation, 684 increased from 150) and **Protocol 4** (Generation of Founders, 3324 embryos from 250) and **Protocol 5** (Breeding and Maintenance of Genetically altered animals [mild], 8666 from 600).

(b) The **moderate** severity procedure in **Protocol 6** (Breeding and Maintenance of Genetically altered animals [moderate], 13872 from 1200).

Category II (experimental procedures)

Protocol 7 (Ageing and phenotyping [Gaucher-related], 1000 from 250); **Protocol 8** (Ageing and phenotyping, 4776 from 800); **Protocol 10**, (Therapeutic interventions for Krabbe disease, 576 from 400 mice); **Protocol 11** (Inoculation of neoplastic or Gaucher-related cells, 300 from 100 mice).

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

Project Licence Standard Condition 18 reports were submitted: (i) 02-FEB-2021; (ii) 22-APR-2022; (iii) 30-JUN-2022. All relate to Gaucher disease in Protocol 12, ('Testing interventions for Cancers' - moderate).

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

Not applicable

5.4 Compliance Assurance Meetings [CAMs] (if applicable):
Provide the date, a summary of the nature of any CAMs incident and the outcome, if known (or if appropriate since the licence was last reviewed).

Not applicable

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

Staffing pressures over weekends and the electronic records completed by technical staff on new management system may lead to errors being made and reflect the challenges that are inevitable when continuity is of importance.

A final point which might affect management for Licence Holders who are required to complete a retrospective review before applying for another Licence: the new electronic Return of Procedures forms are complex but do not record experiments carried out under each protocol separately. This impedes accurate completion of the Retrospective Reviews.

6 Retrospective Review:

Project Licence Holder check list:

AWERB requires the following documents	**Documents sent to AWERB**
Completed copy of this form	Yes as a pdf (30-Jan-2023)
Copies of Condition 18 reports (if applicable)	Yes
Copies of 3Rs publications (if applicable)	Not applicable.

For UBS office use only:

	Date
1. Retrospective review form sent to project licence holder	28/09/21
2. Retrospective review form received by the Deputy Director	
3a. Project Support Team meeting	
3b. 3Rs committee	
3c. AWERB Standing Committee	
4. Process completed	