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:	Dr Maria Alcolea	
Contact details: Address & tel.	L02-R11 Cambridge Stem Cell Institute Jeffrey Cheah Biomedical Centre Cambridge Biomedical Campus University of Cambridge Puddicombe Way Cambridge CB2 0AW	
Project No.	PP7037913	
Project title	Epithelial stem cell behaviour away from homeostasis; translational relevance.	
Project purpose	To investigate how the cells of the oesophagus (the gullet; the tube that connects the mouth to the stomach) and other related tissues (like the skin) adapt to perturbations such as aging, wounding and tumour formation.	

Date licence was granted	28th June 2021
Date of retrospective review is sent to the PPLh	01st April 2025

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	✓	My licence is due to expire in 6 to 12 months time and I have no plans to write a new licence
B		My licence is due to expire in 12 to 15 months time and I plan to write a new project licence to continue my research
C		I plan to request the revocation of my licence before its natural expiry date
D		I will no longer be the project licence holder but another person will continue to hold the licence. **The name of the new licence holder will be**:
E		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. For any animals that experienced a higher level of severity than that of the protocol please highlight these figures in **bold.** 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	300	1st year			7			7
				2nd year						0
				3rd year						0
				4th year						0
				Total	0	0	7	0	0	7
2	Mice	Moderate	300	1st year						0
				2nd year						0
				3rd year						0
				4th year						0
				Total	0	0	0	0	0	0
3	Mice	Moderate	50	1st year						0
				2nd year						0
				3rd year						0
				4th year						0
				Total	0	0	0	0	0	0
4	Mice	Mild	30000	1st year			831	**11**	**15**	857
				2nd year			1646	**43**	**50**	1739
				3rd year		3	1887	**44**	**45**	1979
				4th year			2364	**53**	**21**	2438
				Total	0	3	6728	**151**	**131**	7010
5	Mice	Mild	1500	1st year			32			32
				2nd year			25			25
				3rd year			15			15
				4th year			4	**2**		6
				Total	0	0	76	**2**	**0**	78
6	Mice	Moderate	1500	1st year			12	9	**2**	23
				2nd year			33	30	**8**	71
				3rd year			56	38	**2**	96
				4th year			63	110	**1**	174
				Total	0	0	164	187	**13**	364
7	Mice	Moderate	3000	1st year			13	49	**22**	84
				2nd year		10	29	164	**3**	206
				3rd year			9	201	**2**	212
				4th year			94	60	**1**	155
				Total	0	10	145	474	**28**	657

8	Mice	Moderate	1500	1st year				21		21
				2nd year				29	**1**	30
				3rd year			9	55		64
				4th year				4		4
				Total	0	0	9	109	**1**	119
									Whole Total	8235

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

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

Protocol number	Severity higher/ same/ lower than anticipated	Reason
4	Higher	Our breeding protocol has shown moderate cases (151/7010). These constitutes sporadic cases (2.15%) of Malocclusion, Hydrocephalus, Fight wounds, and repeated earclipping (for genotyping). We have also found severe animals (131/7010), accounting for 1.87% of all animals under breeding and maintenance. Majority of them were reported as “found dead”. There were 16 animals culled sick, mainly due to parturition distress.
5	Higher	There were 2 moderate cases, which we believe due to peritonitis upon Tamoxifen injection (sc). This represents 2 mice out of 78 treated with tamoxifen in the entire licence, accounting for 2.56%.
6	Higher	13 out of 364 mice experienced severe severity, accounting for 3.57%. 6 were cannibalised pups shortly after injection, 6 adults had adverse effects after tam administration and one ageing animal showed weight loss (no procedure linked).
7	Higher	28 out of 657 mice experienced severe severity, accounting for 2.46%. Our long experience with the early tumour DEN model allowed us to nicely predict the severity of this protocol. DEN carcinogen treatment is well tolerated by the majority of mice. We have now noticed that DEN treated animals also develop sporadic liver damage as they age. Working closely with animal technicians we have identified that increased size and hardening of the abdomen can be used as a clinical sign to define the endpoint, and avoid welfare deterioration.
8	Higher	1 out of 119 mice experienced severe severity, accounting for 0.84%, it falls within the 1% anticipated in the adverse effects for this treatment.

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).

Although the majority of adverse effects were well anticipated due to our extensive experience with the models and protocols under study, a number of unanticipated complications have arisen over the course of the project.

Protocol 4 (Breeding and maintenance of genetically modified mice)

The Fucci2a strain has previously shown issues with runt pups and/or cannibalisation. While the strain is typically maintained through homozygous breeding, we have introduced a wild-type animal to increase genetic variability through a phase of heterozygous breeding before returning to a homozygous strategy. We are hopeful this will lead to an improvement. To further mitigate these issues, we plan to implement closer monitoring to allow early identification of poor pup health, with appropriate humane endpoints in place. The breeding strategy will be revised to enhance genetic diversity. (See 1 SC18 report attached)

SC18 report: 17.09.2021

Protocol 6 (Changes in epithelial cell behaviour over time)

Some animals showed intolerance to tamoxifen, an effect observed at low frequencies across various strains. To address this, we will implement more frequent monitoring to ensure early detection of poor health, triggering humane endpoints where necessary. Additional visual checks will be carried out on at-risk animals, and samples will be collected sooner to limit potential suffering. We will implement the following measures:

-To avoid the development of tamoxifen related wounds, repeated injections will be performed on different anatomical sites (such as right and left flank, or nape). In case of wounds or accumulation of oil into small masses, those will be monitored over time.

-Animals scheduled to undergo repeated tamoxifen administration by subcutaneous administration will undergo claws clipping before the procedure to limit potential scratching on the site.

-Skin irritation, oil accumulation or small masses can be contained by NSAID oral administration.

In some cases, pups injected with tamoxifen were cannibalised by one of the breeders. We are currently investigating whether this may be due to developmental delays associated with the phenotype induced by tamoxifen in this line. (See 8 SC18 reports attached)

SC18 reports: 29.07.2021, 29.03.2022, 15.06.2022, 24.06.2022, 24.10.2022, 25.07.2024, 07.07.2025, 07.08.2025

Protocol 7 (Changes in epithelial cell behaviour in response to tissue perturbation)

We have observed that DEN-treated animals occasionally develop liver damage as an adverse effect. This may explain a proportion of unexpected deaths at later experimental time points. This finding has been reported to the Home Office. (See 5 SC18 reports attached). Our ongoing PPL amendment now considers additional measures to monitor animals treated with carcinogens and a detailed scoring system with associated end-points..

SC18 reports: 19.05.2022, 16.01.2022, 28.03.2022, 30.03.2022, 16.07.2025

Protocol 8 (Epidermal wounding)

We constantly work with our NVS, NACWO and Animal support staff to refine our procedures, including this surgical protocol. This has results in the continuous improvement of the outcome and tolerance of our surgeries. Among the two SC18 reports submitted, one case involved an unexplained death, and the other was a keep-alive request. Following consultation with the NVS, we are preparing an amendment to this protocol to more accurately record the adverse effects of the protocol. (See 2 SC18 reports attached)

SC18 reports: 13.08.2022, 15.01.2025

Overview

All experimental procedures and adverse effects are recorded in our MCMS database by either animal technicians or research scientists. If entered by technicians, the data are forwarded to the PIL responsible for the specific experiment or mouse line to maintain independent oversight. All incidents are ultimately reported to me as the PPL holder. Each case is reviewed individually, and any follow-up actions are documented in MCMS. Independent records are also maintained by the relevant PIL scientists and are accessible to the entire research group.

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
4	8.16% = Combination of 456 animals culled sick (6.50%, parturition distress, fitting/seizure, deformed, malocclusion, runted), and 116 found dead (1.65%)
6	6.32% = Combination of 11 animals culled sick (3.02%, moribund, fight wound), and 12 found dead (3.30%) due to Tamoxifen tolerance.
7	5.78% = Combination of 35 animals culled sick (5.33%, weight loss, fight wound, eye opacity), and three found dead (0.46%) due to Tamoxifen and DEN tolerance.
8	1.68% = Combination of one animal culled sick (0.84%, moribund), and one found dead (0.84%)

2.5 Were your estimated animal 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	7/300. Less superovulations were needed to establish our colonies.
2	0/300. Embryo recipients were not needed to establish our colonies.
3	0/50. Vasectomies were not needed to establish the colonies.
4	7010/30000. This is primarily due to the uncertainty and disruptions caused by the post-pandemic situation at the start of the project, which significantly slowed progress. However, we have since been awarded multiple funding grants and are expanding

	our research team. As a result, we expect to increase usage in the remaining project period, though it is unlikely we will reach the originally proposed total of 30000 mice.
5	78/1500. This protocol was initially designed for lineage tracing experiments and cell labelling. However, as the various projects have evolved, our focus has shifted primarily toward single-cell RNA sequencing, with the intention of conducting lineage tracing in specific subpopulations of interest at a later stage. We plan to pursue these experiments in the upcoming PPL term. The analysis to identify the cell types underlying our observations is still in progress.
6	364/1500. This protocol was originally developed to enable lineage tracing and cell labelling, with a particular emphasis on incorporating aging as a key variable in the study design. As the projects progressed, we prioritized single-cell RNA sequencing to capture the molecular signatures of aging across different cell types. Lineage tracing in selected age-associated subpopulations remains a future objective, which we plan to pursue in the next PPL term. Our analysis to identify the specific cell types and pathways involved in age-related changes is still ongoing.
7	657/3000. This protocol was initially designed to support lineage tracing and cell labelling, with a specific focus on studying tissue perturbation using the DEN carcinogen model. As the project evolved, we concentrated on single-cell RNA sequencing to better understand the cellular responses to DEN-induced injury and transformation. Lineage tracing within subpopulations affected by DEN exposure remains a key objective for future work, which we plan to carry forward in the next PPL term. The analysis aimed at identifying the cell types and molecular pathways driving DEN-induced changes is still in progress.
8	119/1500. This protocol was originally included based on the expectation that most projects in the laboratory would require validation through cell transplantation into the skin. While this step is still anticipated once ongoing analyses are complete, many of our projects have not yet progressed to that stage, resulting in lower-than-expected usage so far. However, as several projects are now approaching completion, we anticipate a significant increase in the use of this protocol. To better align with our evolving research goals, we have submitted an amendment that will refine the protocol and support expanded use. Consequently, we expect to use more animals in the near future as these studies move forward.

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

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

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

2.7 Has there been any animal wastage? **YES**

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:

In my laboratory we tend to reduce the number of animals used by using productive breeding strategies. For example, by breeding homozygous animals where possible, and good experimental design to anticipate the most efficient breeding strategy (number and length of matings needed).

We also attempt to make use of the maximum number of animals, trying to waste the least possible. For this, we use mice with unwanted genotype and old breeders for *in vitro* pilot experiments where compatible.

Despite all our efforts, we still produce a number of animals that we are unable to make use off. Those include unwanted genotypes (if not compatible to be used for other applications), and animals that are culled surplus, if not from a mouse line relevant for our *in vitro* work (in which case it would be used for tissue culture).

Estimated number or percentage of animals wasted and if appropriate provide this information by protocol:
All cases of unwanted genotype come from protocol 4 (breeding and maintenance), accounting for a total of 20.54% (1692 out of 8238 mice).

Justifications for Unwanted Genotype

- A low copy number of K14-Cre is intentionally maintained to reduce the incidence of eye problems in mice; consequently, many experimental mice do not carry the K14-Cre allele.
- Sex-based selection for sequencing experiments results in males being prioritized while females are often classified as unwanted genotypes (initially counted as surplus but now being re-fated).
- The re-derivation from cryopreservation during 2021–2022, in preparation for the AMB move, required multiple crosses to regenerate HOMs, increasing unwanted genotype numbers.
- Outbreeding efforts for some lines, such as MACS (to breed out the Eng2F gene) and removal of resistance cassettes, also contribute to unwanted genotypes.
- Some mutation lines have to be kept in heterozygosity to prevent tumour formation that happens in homozygosity. However, homozygous animals are needed for experiments. So, a significant portion of animals produced will come with an undesired genotype.

Majority of culled surplus cases come from protocol 4 (breeding and maintenance), with the exception of 1 animal from protocol 6 (Changes in epithelial cell behaviour over time), accounting for a total of 15.89% (1309 out of 8238 mice).

Justifications for Culled Surplus

- Delays in routine tasks such as ear-clipping, obtaining biopsies for genotyping, and cage consolidation at weaning lead to poorly grouped animals, especially males that cannot be reconsolidated, resulting in surplus.
- Male aggression causes some males to be culled, which is undesirable and adds to surplus.
- Trio breedings aimed at reducing inbreeding, produce more pups than needed to maintain a line.
- Certain lines require periodic refreshing, for example, MAAC (mTmG) and MABK (H2BGFP).

Ongoing Improvements

- Lines rarely used have been systematically closed, for example, MABF (Fucci2a).

- Some HIF1a-related lines (MACN, MACO) were recruited for experiments but discontinued after unsuccessful testing.
- Recently obtained mutation lines, introduced late in the current PPL, are under active review for improvement opportunities.

2.8 For licences that authorise the breeding and maintenance of genetically altered animals:
a) explain how you optimise breeding efficiency, and
b) explain what use is made of any excess stock animals, and
c) explain if, and how, you have used any transgene repository – national, international, or internal.

a) Optimizing Breeding Efficiency

We optimise breeding efficiency by closely aligning mouse production with experimental needs, avoiding unnecessary surplus and minimising wastage. Our approach is proactive and flexible, tailored to each mouse line's characteristics and reproductive performance. Close collaboration within our group allows us to synchronise breeding timelines with project needs, reducing overproduction and enhancing overall breeding efficiency.

i) **Targeted Breeding:** We breed genetically modified mice specifically for planned experiments. This allows us to reduce excess animals and ensures that colonies are only maintained as needed.
ii) **Maintenance Breeding Strategy:** For non-experimental lines used primarily for maintaining genetic backgrounds, we keep a stock of two cages each of males and females. When these stocks reach 16 weeks of age, they are replaced through planned matings to ensure continuous availability of healthy breeders.
iii) **Homozygosity:** When feasible, lines are maintained as homozygous to maximise the proportion of usable offspring, increasing breeding efficiency for both experimental and maintenance purposes.
iv) **Genetic Integrity:** To minimise genetic drift, we refresh the genetic background by backcrossing to the original inbred strain approximately every 10–15 generations.
v) **Experimental Planning:** Breeding for experiments is carefully calculated based on litter size and genotype ratios. We estimate the number of litters needed and include a 20% buffer to accommodate variability. Excess animals are typically incorporated into further experimental validation, reducing unnecessary culling.

b) Use of Excess Stock Animals

We make every effort to ensure that surplus animals are used meaningfully and are not wasted. we ensure that all animals are used to their fullest potential in line with the principles of the 3Rs.

i) **Institutional Tissue Sharing:** Surplus animals or those with unwanted genotypes are made available to other researchers within our institute through an internal tissue-sharing list. This promotes efficient use of resources and supports collaborative research.
ii) **Experimental Validation:** Where compatible, excess animals are used to perform additional validation experiments, particularly in response to new or evolving data. This helps strengthen research findings without the need for additional breeding.
iii) **Pilot Study:** Surplus animals may also be used in pilot experiments, provided the resulting data would remain valid if the pilot is successful. This supports exploratory research while minimising animal use.
iv) **Control Groups:** Wild-type or genotype-compatible surplus animals are often used as controls in ongoing experiments, reducing the need for additional breeding of control animals.

v) ***In Vitro* Work:** A significant portion of excess animals is used for *in vitro* studies, where genotype compatibility allows. This represents our primary avenue for utilising surplus stock, particularly in early-phase or mechanistic investigations.

c) Use of Transgene Repository

We store genetic material, such as cryopreserved embryos or sperm, in internal transgene repository. This not only protect against the potential loss of specific strains but also allowing us to regenerate lines when needed without having to continually breed large numbers of animals.

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

a) accessed by other researchers at the University? **YES**
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 with an MTA applied? **YES**

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

We are open to collaboration and encourage other researchers to use our strains, provided that their research aligns with the ethical guidelines and animal welfare standards. Animal stocks and procedures are recorded on MCMS. This data is accessible to other members of the university using MCMS.

External researchers may access our strains through a MTA, ensuring that the use of the strains complies with legal, ethical, and intellectual property considerations while fostering academic and scientific collaboration. Please note, some internal sharing of strains, may also be subject to relevant MTAs.

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

MCMS is not available to researchers outside the University due to security reasons.

2.10 Have you been able to refine and/or reduce the impact of contingent harms arising from your use of animals (contingent harms are harms for example that arise from the transportation of animals, single housing animals or housing animals in non-Code of Practice accommodation, etc.). **YES**

Please explain/provide examples.

Yes, we actively implement strategies to refine our practices and reduce contingent harms, with a strong focus on animal welfare throughout the research process. Our refinements address housing, handling, and experimental design:

i) **Housing and Socialisation:** All animals are housed in accordance with the standards outlined in the Code of Practice. To minimise stress from isolation, we carefully plan our experimental timelines to allow for the consolidation of animals into appropriate social groups. When single housing is unavoidable, such as for specific experimental needs or aggressive animals, these individuals are closely monitored for signs of distress, and enrichment is provided where possible.

ii) **Minimising Transport Stress:** Since the majority of our genetically modified lines are bred in-house, transport-related stress is minimised. Animal transport is limited to essential cases, such as the introduction of new lines or procurement of wild-type controls. In those cases, transportation is carefully coordinated to ensure compliance with welfare standards.

iii) **Reducing Excess Animals:** We proactively reduce the number of surplus animals—especially those with incorrect genotypes—by repurposing them for relevant studies. This includes using them as controls, in pilot experiments, or in *in vitro* studies, provided their genetic background is suitable. This approach aligns with the principle of Reduction under the 3Rs framework.

iv) **Refining Genotyping Practices to Prevent Isolation:** To reduce the risk of single housing, we perform genotyping before animals reach three weeks of age whenever possible. This early identification allows animals to be grouped by sex and genotype while still juveniles, reducing both social stress and the risk of aggression that can arise when older animals are grouped.

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

Species	Source	Approx. percentage
Mice	In-house breeding	93.6%
Mice	Charles River	6.14%

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

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

Protocol No.	Original estimated Number	Amended estimated number

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

NA

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?

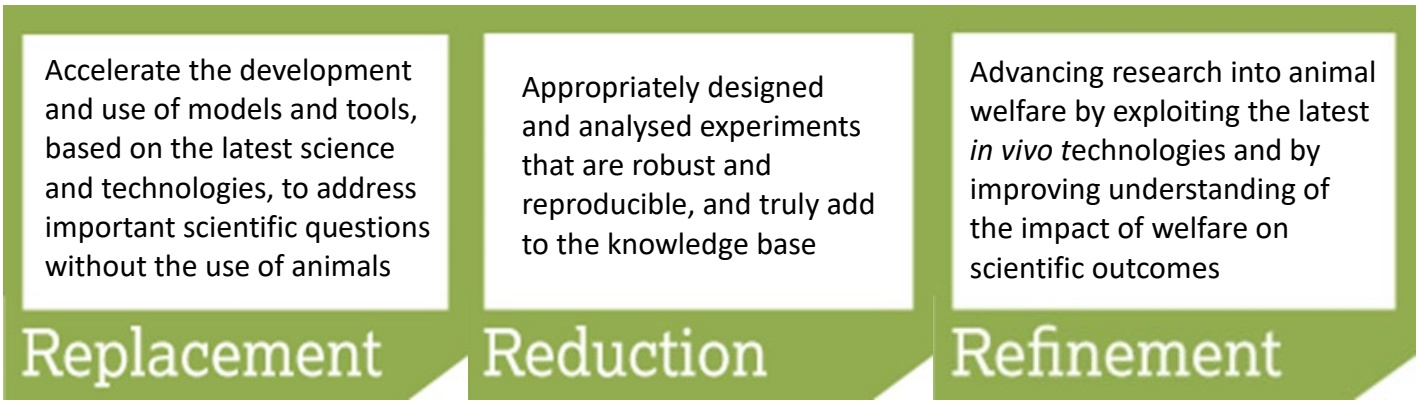
NA

3. **3Rs:**

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

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

Definitions



3.1 Have you attempted or been able to replace any of your techniques or models with non-animal alternatives? **YES**

Please explain.

Given the complexity of cell communication and cooperation in tissue regeneration and cancer, animal work remains necessary for certain aspects of our research. However, we have developed a 3D organ culture model that mimics *in vivo* cell behaviour, tissue regeneration, and tumour formation. We have developed methods to grow both epithelial tissues (such as skin and oesophagus) and the supporting stromal cells outside the animal. This approach serves as an outstanding tool for replacing animal use in short-term experiments focused on wound healing and tumour formation. At present, all our projects make use of this technique wherever possible.

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)? Please give an indication of the actual numbers reduced.

Yes, we have implemented a range of strategies that have substantially reduced our use of animals, without compromising scientific rigour. These strategies include alternative models, refined experimental design, and innovative analytical approaches:

i) **Efficient Breeding Strategies:** Where possible, we maintain mouse lines as homozygous, ensuring that nearly all offspring carry the desired genotype. This increases the proportion of animals suitable for experiments and reduces breeding waste.

ii) **Statistical Design and Collaboration:** We collaborate closely with theoretical biophysicists (notably Prof. Ben Simons' group) to optimise our experimental design. These collaborations

ensure that experiments are powered appropriately, using the minimum number of animals required to achieve statistically meaningful results.

iii) **Precise Line Management:** Animals are bred strictly according to experimental needs. Excess animals (where unavoidable) are repurposed for *in vitro* cultures, validation, or pilot studies if genetically compatible. This coordinated approach is a critical tool for reducing overall animal use.

iv) **3D Organ Culture Model:** One of our most impactful refinements has been the implementation of a 3D organ culture system. From a single oesophagus, we can generate enough cells to test three separate experimental conditions, reducing the number of animals used per experiment by approximately one third. These cultures can be expanded and maintained for up to a year, significantly decreasing the number of animals needed in long-term studies.

v) **Lineage Tracing at Single-Cell Resolution:** By using *in vivo* lineage tracing, we track hundreds of labelled cells per tissue in each animal. This method allows us to use as few as three animals per condition while generating robust, high-resolution data. Theoretical modelling helps predict cell behaviour, which can then be validated in targeted follow-up experiments—further reducing the need for exploratory animal use.

vi) **Minimal Tissue Requirement Techniques:** We employ advanced imaging and molecular techniques that require extremely small tissue samples (as little as 1 mm²). This allows us to carry out multiple analyses on a single tissue, rather than using multiple animals.

vii) **Skin Transplantation:** Our skin graft protocols permit the inclusion of internal controls within the same animal by using localised treatments. This improves data quality and statistical robustness while reducing the number of animals needed.

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

Please explain:

A network of collaborators within the UK is maintained. Particular mouse strains are exchanged to reduce animal imports, minimise additional breeding, and prevent surplus generation. Tissues are also shared with collaborators investigating other anatomical regions. Furthermore, we are registered in the institutional animal tissue sharing list to support broader sharing efforts.

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.

Reproductive Techniques

- Superovulation induction via:
  - Intraperitoneal injection (AA)
  - Subcutaneous injection (AA)
- Surgical and non-surgical embryo/blastocyst implantation (AB / AA-AB)
- Vasectomy (AB)
- Breeding and generation of genetically altered offspring via:
  - Conventional breeding
  - *In vitro* embryo manipulation and implantation

Tissue Collection and Genotyping

- Tissue biopsy for genotyping via:
  - Ear biopsy (AA/AB)
  - Blood sampling (AA/AB)

- Hair sampling (AA/AB)
- Mouth swabbing (AA/AB)

Gene Induction and Substance Administration

- Gene induction using agents (e.g., tamoxifen, doxycycline) via:
  - In diet/oral (AA, post-weaned only)
  - Subcutaneous injection (AA)
  - Topical application (AA)
- Administration of substances affecting cell behaviour via:
  - Diet or drinking water (AA, post-weaned only)
  - Oral gavage (AA)
  - Subcutaneous injection (AA)
  - Topical application (AA)
- Cell labelling with agents (e.g., EdU, BrdU) via:
  - Diet/drinking water (AA, post-weaned only)
  - Oral gavage (AA)
  - Subcutaneous injection (AA)
  - Intraperitoneal injection (AA, post-weaned only)
  - Topical application (AA)
- Tissue perturbation using agents (e.g., carcinogens) via:
  - Diet/drinking water (AA, post-weaned only)
  - Oral gavage (AA)
  - Subcutaneous injection (AA)
  - Intraperitoneal injection (AA, post-weaned only)
  - Topical application (AA)

Sampling and Euthanasia

- Blood sampling from superficial vessel (AA/AB)
- Urine collection via non-invasive methods (AA)
- Terminal blood sampling (AC)
- Killing by a Schedule 1 method

Skin Procedures

- Skin wounding (AB) via:
  - Full-thickness excision biopsy (up to 10 mm)
  - Incision (up to 10 mm)
  - Wounds protected with aseptic bandaging (may be reapplied once)
- Skin transplantation (AA/AB/AB):
  - Subcutaneous injection
  - Intradermal injection
  - Surgical grafting into wounded area
  - Cell transplant in solution or matrix (e.g., Matrigel), with optional use of silicon chambers

Animal models

- 26R-nT-nG
- AhCre-Confetti
- AhcreERT
- AhCre-mT/mG
- AhCreYFP
- Athymic Nude Mice
- Col1a2CreERT
- Col1a2crexR26Conf
- Col1a2YFP

- confetti K14
- Const-H2BGFP
- DNMAML1
- DNMAML1xK14CreERxKrasxp53
- DNMxp53xK14Cre
- DOBcreYFP
- FGFR1
- FGFR1fx/FGFR1d/K14Cre/mTmG
- Fucci2a-MPA
- H2BGFP (Efu)
- K14Cre JMG
- K14CreERxKRasxp53-Hom
- K14CreERxKrasxp53xmTmG
- K14CreERxmT/mG
- K14CreERxmT/mGxDNMAML1
- KLF4flox
- KLF4flox-K14Cre-mTmG
- Lgr5 CreER
- Lgr5-CreERTxmTmG
- mTmG
- mTmG/nTnG
- mVenus-p27K
- PB-CagGFP
- Pdgfra-cre x R26-YAP5SA
- PDGFRa--GFP
- PDGFRa--GFPxmTmG
- PDGFRa--GFPxnTnG
- PGC-DTR
- R2(E47M)
- R2(E47WT)
- R26Confetti
- R26CrexRosa Td Tomato
- R26-Fucci2a
- R26Tet
- R26TetxH2BGFP
- Red2KRas
- Red2Yap x K14Cre
- Rosa tdTomato
- Rosa26Cre x Confetti
- RosaCre / RosaConfetti
- RosaCreERT2 confetti
- Rosa-CrexmT/mG
- Rosa-Stop-Confetti
- Rs26 YFP CRE
- RYFP
- Sftpc-CreERT2
- Sox9_CreERT2 YFP
- Sox9flox
- Sox9flox-K14Cre-mTmG
- SOX9-mT,G
- Stathmin-CreERT2

- Tnfrsf19/YFP
- C57/B6
- YAP1 flox

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

The techniques and models have been successfully used in previous work and are well-established within the field. Based on our experience, they consistently produce reliable and reproducible results aligned with the scientific objectives. These techniques are optimised for effective outcomes with minimal adverse effects.

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

Some limitations encountered include variability in response to gene induction or substance administration, likely due to individual differences in absorption or metabolism. These were addressed by optimising dosage and timing. Animal numbers were determined in consultation with expert statisticians to ensure the experiments are well-powered and scientifically robust.

Minor swelling or delayed healing has occasionally occurred following grafting procedures. These issues were effectively managed with refined surgical techniques, aseptic handling, and appropriate post-operative care.

Transient weight loss has also been observed after certain treatments. Through close monitoring and adjustments to timelines, animals typically recover fully without affecting welfare or data integrity.

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.

Substance administration for cell labelling and tracking is based on established protocols and long-term expertise. We consistently use the lowest effective dose, minimising any potential harm.

In studies using carcinogens to explore early tumour development, we have optimised dosing over the past decade to use low, non-invasive delivery (via drinking water). Most animals remain healthy for over a year with no complications.

For injury and skin procedures, we have significantly refined our methods over the past five years. We now use clinical-grade punch biopsy tools to create small wounds that heal faster and cause less discomfort. Graft sizes have been reduced to a maximum of 1 cm, and the use of Tegaderm transparent medical dressings has greatly improved post-operative care. This dressing provides protection while allowing animals to move freely and facilitates easy visual monitoring of the wound, reducing handling and stress. Animals typically remove the dressing themselves by day 4–5, at which point wounds are stable and healing well.

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

If not, why?

NA

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 modifications have been made. We use a structured welfare monitoring system that includes a scoring sheet and Mouse Grimace Scale to assess pain and discomfort more accurately. Animals are also monitored for clinical signs such as hunched posture, piloerection, and subdued behaviour. A combination of weight records and clinical signs are used to decide on the humane endpoint as described in the adverse effects. Animals are monitored daily, with increased frequency during and after procedures such as surgery, substance administration, or carcinogen exposure.

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 endpoints detailed in the project licence have been appropriate and effective. Side effects from procedures are monitored through regular weighing, daily health checks, and scoring sheets. This system allows us to detect any signs of distress early and apply Humane Endpoints as needed.

So far, we have not needed to modify these endpoints, as animals undergoing procedures under this licence have generally not shown signs of suffering. In rare cases where an animal appears unwell, we increase monitoring frequency and administer pain relief if appropriate. If the condition does not improve, the animal is humanely euthanised to prevent unnecessary suffering.

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.

No non-schedule 1 method will be employed.

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

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.

NA

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?

NA

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).

I do agree. Information can be added to the UBS 3Rs Search Tool.

4. Scientific Progress and Benefit Achievements:

4.1 Taking into consideration that your licence may not be due to expire, has your research progressed as you expected? **YES, other than the indicated delays**

4.2 When your licence is due to expire will you have completed the programme of work described in this licence? **YES, we would have addressed our original questions, however the majority are still ongoing work.**

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

Objective 1: Identify changes in the regenerative response of epithelial tissues as tissue ages.

Achievement / Discovery:

The main focus of my laboratory has been to study epithelial cell fate transitions. Funded by a Wellcome Trust/Royal Society Sir Henry Dale Fellowship (SHDF), as well as MRC, CRUK and Worldwide Cancer Research grants (among others), we have been able to investigate the mechanisms governing how cells change their behaviour in response to a variety of physiological or pathological perturbations away from homeostasis, including development, regeneration, and early tumour formation.

Following on my previous work, which showed that the mouse oesophagus represents an uncomplicated system, suitable to understand shared processes across different squamous epithelial tissues, my laboratory has remained focused on studying the epithelium of the oesophagus. We implement an interdisciplinary approach, combining our expertise in in vivo quantitative lineage tracing and 3D organ cultures with new techniques developed in my laboratory. These include, low input single-cell RNA sequencing (scRNA-seq) and proteomics, ectopic 3D organ cultures, as well as in vitro tools to manipulate the mechanical properties of squamous tissues.

We have investigated cell fate transitions in the mouse oesophageal epithelium during the postnatal period and tissue regeneration. We have demonstrated that epithelial cell fate transitions are regulated by the balanced contributions of two transcription factors KLF4 (McGinn, Nature Cell Biology, 2021) and SOX9 (Bejar et al, BioRxiv; currently being considered for publication in Nature Communications) The working hypothesis, which represents a fundamental part our programme of work, poses that the balance between these two transcription factors defines a switch from proliferative expansion to balanced self-renewal and differentiation, marking the outcome of a particular cell fate transition.

Objective 2: Define changes in the early tumour microenvironment relevant for tumour formation and progression.

Achievement / Discovery:

Using our established early tumour model (Alcolea *et al.*, 2014; Frede *et al.*, 2016), we have studied the influence of the mesenchymal compartment on epithelial behaviour at the onset of tumourigenesis. Employing the DEN-induced oesophageal tumour model, which mimics the mutational landscape of the aging human oesophagus (Colom *et al.*, 2020), we have combined lineage tracing with detailed 3D histological analysis. We have discovered that, at the very earliest stages of tumour formation, mesenchymal cells undergo significant reorganisation to form a supportive niche. This activated microenvironment appears to facilitate the expansion of mutant epithelial cells. Our findings provide new insight into how early mesenchymal-epithelial crosstalk contributes to tumour initiation. A preprint has

been submitted to bioRxiv, and the manuscript is currently under revision in Nature (Skrupskelyte et al, BioRxiv, 2024).

Objective 3: Reveal whether cells re-acquire developmental features during tissue regeneration or tumorigenesis.

Achievement / Discovery:

In efforts to understand epithelial plasticity under non-physiological conditions, we explored whether adult epithelial cells could undergo transdifferentiation when exposed to ectopic environmental cues. We found that mouse oesophageal progenitor cells can be reprogrammed into hair follicle cells when transplanted into the dermal environment of the skin. Within 10 days, these cells begin forming *de novo* hair follicle-like structures, expressing canonical follicular markers. Remarkably, this process can also occur *in vivo* following heterotypic transplantation, with oesophageal-derived hair follicles maintained long-term. These results demonstrate that adult epithelial cells can re-acquire developmental-like plasticity in response to foreign niche signals, both *in vitro* and *in vivo* (Bejar et al, BioRxiv; currently being considered for publication in Nature Communications).

Objective 4: Uncover the impact of tissue challenges, including development, aging, and injury, for tumour formation.

Achievement / Discovery:

Our work across multiple models has revealed that both physiological (e.g., development and aging) and pathological (e.g., injury or carcinogen exposure) challenges can modulate epithelial plasticity and tumour susceptibility. For example, mechanical strain during development induces a controlled shift in progenitor cell behaviour (see Objective 1), while carcinogen exposure (see Objective 2) triggers mesenchymal reprogramming that supports early tumour growth. Furthermore, by modelling extreme tissue perturbation through heterotopic transplantation (see Objective 3), we demonstrated the capacity of adult epithelial cells to undergo identity changes, suggesting that environmental stressors can unlock latent plasticity. These findings collectively underscore the profound impact of developmental, mechanical, and oncogenic challenges in shaping epithelial fate and tumour potential. The molecular mechanisms modulating the response of epithelial cells upon different perturbations, represented the focus of my laboratory in the immediate future.

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.).

One focus of our study was to investigate clonal competition between different mutant populations. However, during our initial trial, we observed unexpected mass formation in some animals. This outcome was not anticipated under the current protocol. As a result, we were unable to proceed with the planned experiments until the observation could be better understood and addressed within an ethically and scientifically appropriate framework. We have currently submitted an amendment to the protocol to allow further investigation of this finding and to ensure we can proceed safely and effectively with this line of research.

4.5 In the expected benefits section of your licence you described the benefits you believed would be likely to arise from your programme of work. Please explain what benefits have actually been achieved

and, if appropriate, explain how these differ from what you anticipated you could achieve when you wrote the licence.

The new information generated through this study has been disseminated via scientific publications and presentations at national and international conferences. These findings primarily benefit the scientific community, particularly researchers working in tissue regeneration and cancer biology, and contribute to the development of future therapeutic strategies that may ultimately benefit patients and healthcare providers.

As part of our standard practice, all relevant datasets and findings are made publicly available through open-access repositories. This ensures that other researchers can access and build upon our work, facilitating broader scientific discovery beyond the scope of our own studies. We help minimise unnecessary repetition of similar experiments in other laboratories, thereby contributing to a reduction in animal use across the wider research community.

Animals involved in our research have benefited directly from our rigorous application of the 3Rs. We have successfully reduced the number of animals used through techniques such as 3D organ cultures and single-cell lineage tracing, which allow for high-quality data collection from fewer animals. Refinements such as early genotyping, improved social housing strategies, detailed welfare scoring systems, and the use of the Mouse Grimace Scale have improved monitoring and reduced the severity of potential harms.

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?

While we were temporarily unable to pursue one of our objectives in studying clonal competition between mutant populations, due to the unexpected mass formation observed in our initial trial, this has not had a significant impact on the overall benefits arising from the project. The other major objectives have been successfully addressed, generating novel insights into epithelial plasticity, developmental transitions, and early tumour microenvironment dynamics. The unexpected findings from the clonal competition trial have, in fact, opened a new line of investigation that may yield additional benefits. We are preparing a protocol amendment to resume this work, and we anticipate being able to address the outstanding question in due course without compromising the overall scientific value and translational potential of the project.

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)?

During the course of this PPL, we have successfully developed and refined several advanced techniques to study epithelial cell behaviour within their native or physiologically relevant environments. These approaches have enhanced both the resolution and relevance of our findings while improving experimental efficiency and reducing animal use. Key developments include:

i) 3D Organ Culture and Tissue Recombination Models

We developed *ex vivo* 3D organ culture systems that allow the recombination of live epithelial and stromal tissues from animals of different genetic backgrounds. This model enables the investigation of how epithelial cells respond to distinct microenvironmental cues in a native-like context. Unlike traditional cell transplantation methods—which often involve invasive

procedures, are limited in biological relevance, and impose a greater animal welfare burden—this technique preserves tissue architecture and function, providing a more refined and ethical alternative for studying epithelial plasticity and cell fate transitions.

ii) Mechanical Stretch Devices for Whole-Organ Culture

We engineered novel stretching devices to investigate the impact of tissue mechanics on epithelial cell behaviour *ex vivo*. These systems apply physiological levels of strain to intact tissues, enabling us to study how mechanical forces drive cell fate decisions, such as YAP activation and KLF4 expression during postnatal oesophageal development. This represents a significant advance over previous models that used single-cell layers on synthetic matrices, which fail to capture tissue-level biomechanics.

iii) Single-Cell Spatial Transcriptomics

To understand the molecular underpinnings of epithelial behaviour in situ, we established single-cell spatial RNA sequencing protocols. These approaches preserve spatial context, allowing us to pinpoint where specific transcriptional changes occur within tissue architecture—something not achievable with conventional bulk or dissociated single-cell methods.

iv) High-Resolution Single-Cell RNA Sequencing on Minimal Tissue

We developed protocols enabling high-quality single-cell RNA sequencing from extremely small samples, sometimes as few as 100 cells. This has proven essential for studying early tumour lesions or rare progenitor populations in developing or regenerating tissues, where sample size is inherently limited.

v) Deep Learning for Image Analysis of Complex Tissues

To efficiently and accurately analyse large imaging datasets, particularly from highly cellular tissues like the oesophageal epithelium, we implemented deep learning algorithms for automated image segmentation and quantification. This has significantly improved throughput and objectivity in data analysis while supporting longitudinal and high-content studies.

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:

Skrupskelyte G*, Rojo Arias JE*, Dang Y, Han S, Bejar MT, Colom B, Fowler JC, Jones PH, Rulands S, Simons BD, Alcolea MP. Pre-cancerous Niche Remodelling Dictates Nascent Tumour Survival. PREPRINT bioRxiv 2024.07.04.602022. Being revised for publication in Nature journal.

Herms A*, Fernandez-Antoran D*, Alcolea MP*, Kalogeropoulou A, Banerjee U, Piedrafita G, Abby E, Valverde-Lopez JA, Ferreira IS, Caseda I, Bejar MT, Dentro SC, Vidal-Notari S, Ong SH, Colom B, Murai K, KingC, Mahbubani K, Saeb-Parsy K, Lowe AR, Gerstung M, Jones PH. Self-sustaining long-term 3D epithelioid cultures reveal drivers of clonal expansion in esophageal epithelium. Nat Genet. 2024 Sep; 56, 2158–2173

Alcolea MP, Alonso-Curbelo D, Ambrogio C, Bullman S, Correia AL, Ernst A, Halbrook CJ, Kelly GL, Lund AW, Quail DF, Ruscetti M, Shema E, Stromnes IM, Tam WL. Cancer Hallmarks: Piecing the Puzzle Together. Cancer Discov. 2024 Apr 4;14(4):674-682

Colom B, Herms A, Hall MWJ, Dentro SC, King C, Sood RK, Alcolea MP, Piedrafita G, Fernandez-Antoran D, Ong SH, Fowler JC, Mahbubani KT, Saeb-Parsy K, Gerstung M, Hall BA, Jones PH. Mutant clones in normal epithelium outcompete and eliminate emerging tumours. Nature. 2021 Oct;598(7881):510-514.

Bejar MT*, Jimenez-Gomez P*, Moutsopoulos I, Colom B, Han S, Calero-Nieto FJ, Göttgens B, Mohorianu I, Simons BD, Alcolea MP. Defining the transcriptional signature of esophageal-to-skin lineage conversion. PREPRINT, bioRxiv. 2021, 431899. Being revised for publication in Nature Communications journal.

Publications pending, or under review:
Skrupskelyte G*, Rojo Arias JE*, Dang Y, Han S, Bejar MT, Colom B, Fowler JC, Jones PH, Rulands S, Simons BD, Alcolea MP. Pre-cancerous Niche Remodelling Dictates Nascent Tumour Survival. PREPRINT bioRxiv 2024.07.04.602022. Being revised for publication in Nature journal.

Bejar MT*, Jimenez-Gomez P*, Moutsopoulos I, Colom B, Han S, Calero-Nieto FJ, Göttgens B, Mohorianu I, Simons BD, Alcolea MP. Defining the transcriptional signature of esophageal-to-skin lineage conversion. PREPRINT, bioRxiv. 2021, 431899. Being revised for publication in Nature Communications journal.

Presentations at scientific meetings:

1. European Association for Cancer Research (EACR) Annual Meeting (Lisbon, Portugal). Title: **More than Mutations; a Survival Guide for Emerging Tumours**. 16-19th June 2025.
2. GRC conference- Epithelial differentiation and keratinization (Ventura, California, US). Hosted by Maria Kasper and Elena Ezhkova. Title: **More than Mutations; a Survival Guide for Emerging Tumours**. 1-6th June 2025.
3. Rockefeller University (New York, US). Anderson Center for Cancer Research Seminar Series. Host: Amy Shyer. Title: **Fate versus Free will; how does the environment shape early tumour survival**. 1st Mary 2025.
4. QMUL seminar. London. Host: Gernot Walko. Title: **Fate versus free will; how does the environment shape early tumour survival?** 24th March 2025
5. Medicine at the Crick. THE GOOD, THE BAD, AND THE UGLY: TISSUE SOMATIC EVOLUTION IN AGEING AND DISEASE event. The Francis Crick Institute, London. Title: **Fate versus free will; What determines the survival of nascent tumours?** 13th March 2025.
6. SCI-TIF EVENT. AztraZeneca, Cambridge. Title: **Stem Cell Competition as a Driver of Premalignancy and Cancer**. 24th Feb 2025.
7. Kings College London. Stem Cell Lunch. Hosted by Rocio Sancho. Title: **Fate versus free will; how does the environment shape early tumour survival?** 11th February 2025.
8. Developmental Biology Seminar Series. Cambridge University. Title: **Fate versus free will; how does the environment shape early tumour survival?** 10th October 2025.

9. GRC conference- Stem Cells and Niches. Hosted by Ophir Klein and Kim Jensen. Title: **Epithelial cell fate transitions; from development to homeostasis and back**. 11-16th August 2024.
10. Max Planck Institute for Molecular Biomedicine, Munster, Germany. Hosted by Sara Wickstrom. Title: **Fate versus free will; how does the environment shape epithelial cell behaviour?** 27th June.
11. Physics of Cell Fate Decisions 2024. Hosted by Edouard Hannezo. Title: **From development to tumours; what have we missed?** 13-15th May 2024.
12. Fusion Conference- 2nd Stem Cell - Niche Interactions in the Gastrointestinal Tract Conference. Hosted by Ophir Klein and Leanne Jones. Title: **Epithelial cell fate transitions; from development to homeostasis and back**. 7-10th May 2024
13. ISRB Webinar. Hosted my Mekayla Storer. Title: **From mutations to tumours; what have we missed?** 12th March 2024.
14. Aseica (40th Anniversary). A Coruna, Spain. Hosted by Marisol Soengas. Title**: Early Tumour Survival is Dictated by Nascent Stromal Niche**. 14-16 Nov 2023.
15. Karolinska Institutet. Sweden. Hosted by Maria Genander. Title: **Cell fate transitions; How do adult epithelial tissues respond to perturbations?** 15th June 2023.
16. GRC- Epithelial Differentiation and keratinization. Barcelona, Spain. Hosted by Salvador Aznar-Benitah. Title: **Early Tumour Survival is Dictated by Nascent Stromal Niche**. 4-9 June 2023.
17. EACR virtual. Hosted by Ilaria Malanchi. Title: **From development to homeostasis and back**. 26th April 2023.
18. London Stem Cell Network (LSCN). Crick Institute, UK. Title: **Cell fate transitions; How do epithelial tissues respond to perturbations?** 25th April 2023.
19. GRC- Stem Cells and Cancer. Italy. Hosted by Cedric Blanpain. Title: **Stromal niche instructs early tumour survival**. 14-19 May 2023.
20. SY-Stem Vienna. Austria. Hosted by Stephanie Ellis. Title: **From development to homeostasis and back.** 22-24 March 2023.
21. Institut Pasteur-Paris. France. Hosted by Romain Levayer. Title: **Cell fate transitions; How do adult epithelial tissues respond to perturbations?** 17th March 2023.
22. Idibell-Barcelona. Spain. Hosted by Jorgi Guiu. Title: **Cell fate transitions; How do adult epithelial tissues respond to perturbations?** 3rd Feb 2023.
23. Quantitative dynamics of aging and premalignancy, IRB, Barcelona. Title: **"Stromal niche instructs early tumour survival"**. Invited by Dr. Alejo Dr. Alejo Rodriguez-Fraticelli, Dr. Salvador Aznar Benitah. November 2022.
24. 12TH DEVELOPMENTAL BIOLOGY COURSE: From Stem Cells to Morphogenesis, Institut Curie, Paris. Title: **"From development to homeostasis and back"**. Invited by Prof Allison Bardin. October 2022.
25. Physics of Living Matter Symposium. 16th Edition. **Title: "Epithelial cell fate plasticity; an oesophageal tale"**. Invited by Prof. Kevin Chalut. September 2022.
26. Hydra European Summer School on Stem Cell Biology and Regenerative Medicine. Title: **"Epithelial cell fate plasticity"**. Invited by Prof. Austin Smith and Prof. Kim Jensen. September 2022.
27. Australian Regenerative Medicine Institute, MONASH University. Title: "**Epithelial cell fate transitions; an oesophageal tale**". Invited by Alberto Rosello-Diez. May 2022.
28. Gurdon Institute (Cambridge). Title: "**Epithelial cell fate transitions; an oesophageal tale**". Invited by David Fernandez Antoran. February 2022.
29. Complutense University of Madrid (Spain). Title: "**Epithelial cell fate transitions: development meets homeostasis**". Invited by Maria Salazar. January 2022. I

30. Cell Physics 2021, Saarbrücken (Germany). Title: "**A biomechanical switch regulates the transition towards homeostasis in oesophageal epithelium**". Invited by Sandra Iden. September 2021.

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**

If you answered NO: why?

NA

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?

We have engaged with the general public through interviews and media features in collaboration with Worldwide Cancer Research and Guts-UK. These include coverage in national newspapers, online news platforms, and social media, helping to raise awareness about our research and its impact.
Examples of public engagement include:

- Facebook Video Interview [https://www.facebook.com/share/v/165XL7rqop/]
- The Sun Article [https://www.thesun.co.uk/health/33787007/acid-reflux-blamed-curries-stress-nine-months-to-live/]
- The Argus Article [https://www.theargus.co.uk/news/24983488.bognor-regis-man-turns-cancer-battle-advocacy/]
- Yahoo News Feature [https://uk.news.yahoo.com/man-still-alive-19-years-050000716.html]
- Sussex Express Story [https://www.sussexexpress.co.uk/news/people/my-indigestion-was-actually-deadly-cancer-says-bognor-regis-dad-of-two-who-says-working-together-is-the-key-to-stopping-the-disease-5019259]

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.

We have submitted and amendment to the current PPL (submitted to HO).

Amendments:

1. Tumour Progression Studies: The amendment includes the extension of the study to cover tumour progression, which was previously limited to early-stage tumour studies. This will enable the research team to assess the transition from benign tumours to invasive cancer, critical for understanding the mechanisms of tumour progression and therapeutic strategies.
2. Additional Monitoring: A detailed scoring system for tumour progression has been introduced, along with enhanced monitoring strategies to closely track animal welfare and detect clinical signs early. This includes tumour surveillance, even when tissue outgrowths are not visible to the naked eye.
3. Refinement in Experimental Procedures: The protocols have been updated to allow for oral administration of substances, reducing the number of animals exposed to invasive treatments, thus refining the experimental plan.
4. Expectation of harmful phenotype: Now includes cancer-related mutations for studying tumour progression. The expectation of harmful phenotypes from tumour growth has been acknowledged, with strategies to prevent unnecessary suffering.
5. Skin transplantation: Added objectives related to tumour microenvironment and tissue perturbation. New humane endpoints and monitoring for tumour-related adverse effects have been incorporated.

The current licence only permits the study of early-stage tumours, restricting the ability to assess the long-term relevance of findings for cancer progression. By allowing tumour progression studies, the changes enhance the scientific value of the research by enabling better understanding of tumour progression mechanisms, which can inform new therapeutic approaches for early cancer intervention.

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.

2021
SC18 Ref 210164 Completed
ReportASRU_University_of__Cambridge_PP7037913__ALCOLEA_29.07.2021_sc18
ASRU_University_of__Cambridge_PP7037913__ALCOLEA_17.09.2021_sc18

2022

ASRU_University_of_Cambridge_708866_ALCOLEA_19.05.2022_sc18
ASRU_University_of_Cambridge_PP7037913_ALCOLEA_16.01.2022_sc18
ASRU_University_of_Cambridge_PP7037913_ALCOLEA_28.03.2022_sc18
ASRU_University_of_Cambridge_PP7037913_ALCOLEA_29.03.2022_sc18
ASRU_University_of_Cambridge_PP7037913_ALCOLEA_30.03.2022_sc18
ASRU_University_of_ Cambridge_PP7037913_Alcolea_20220615_sc18
ASRU_University_of_ Cambridge_PP7037913_Alcolea_20220624_sc18
ASRU_University_of_Cambridge_PP7037913_Alcolea_20220813_sc18
ASRU_University_of_Cambridge_PP7037913_Alcolea_20221024_sc18

2024
ASRU_University of Cambridge_PP7037913_Alcolea_20240725_sc18

2025
KEEP ALIVE REQUEST_ASRU_University of Cambridge_PP7037913_ALCOLEA_20250115_sc18
ASRU_University_of_Cambridge_PP7037913_Alcolea_20250707_sc18
ASRU_University of Cambridge_PP7037913_Alcolea_16.07.2025_sc18
Keep Alive Request ASRU_University of Cambridge_PPL_ALCOLEA_20250807_sc18

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).

Date: 6th March 2025

Summary of the nature of non-compliance:
An unintentional breach of licence conditions was identified involving a request to perform an additional ear biopsy under an approved protocol **(protocol 6)** that did not authorize re-biopsying. The procedure was therefore not covered by the existing PPL, constituting a non-compliance with licence terms.

Outcome:
The matter was reviewed internally and reported to the HO, and the licence holder (myself) was informed of the deviation from approved procedures. Clarification was provided regarding the limitations of the current protocol, and steps were taken to prevent recurrence, including reinforcing procedural boundaries with all personnel involved. **No adverse animal welfare outcomes were reported. The incident has been documented and addressed in accordance with institutional and regulatory guidelines.**

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

Date: 22nd January 2025

Incident summary
A mouse showing 12.72% weight loss on 15/01/2025 was monitored daily and given supplements. By 20/01/2025, the mouse had exceeded 15% weight loss, and was kept alive for a further 48 hours, as staff followed endpoints under Protocol 6, step 1. However, the mouse had not yet begun this protocol stage, so the general humane endpoints should have been applied instead, indicating a misunderstanding of which endpoints were appropriate.

Outcome

- ASRU reviewed the report and did not pursue this as non-compliance, noting the animal had supplementary food, close monitoring, and no other clinical signs.
- Recommendations / actions: additional staff training on interpreting study plans and endpoints; researcher reminded to verify animal/study status and seek licence-team advice when unsure; NACWO advised to prioritise follow-up of animal health concerns.

Date: 25th August 2025

Incident summary
On 25/08/2025, altered drinking water was prepared and provided to mice by a non-PIL holder following incorrect training under a husbandry DOP rather than the regulated procedure version. The incident arose from confusion between two similar DOPs, one of which allows limited non-skilled delegation.

Outcome

- No adverse effects were observed in the animals.
- The team will review and amend the husbandry DOP to clarify which tasks can be delegated.

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.)?

In January 2025, we were informed that a liquid nitrogen dewar at JCBC, which was supposed to contain cryovials of sperm or embryos from 13 of our genetically modified mouse colonies (PP7037913), was found at room temperature. At this stage, we anticipate the potential loss of 9 mouse lines, as we do not have live animals or backup stocks elsewhere for these lines.

The primary implication moving forward is that if we need to recreate these lines, we may have to amend the PPL to account for the increased number of animals or additional procedures required for this process.

6 Retrospective Review:

Project Licence Holder check list:

AWERB requires the following documents	**Documents sent to AWERB**
Completed copy of this form	Yes
Copies of Condition 18 reports (if applicable)	Yes
Copies of 3Rs publications (if applicable)	Yes

For UBS office use only:

	Date
1. Retrospective review form sent to project licence holder	01/04/25
2. Retrospective review form received by the Project Support Team	
3a. Project Support Team meeting	
3b. 3Rs committee	
3c. AWERB Standing Committee	
4. Process completed	