Retrospective Assessment of Project Licences

Project Licence Retrospective Assessment

"The overall purpose of retrospective assessment 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 Ziad Mallat	
Contact details: Address & tel.	Department of Medicine Division of Cardiovascular Medicine VPD-HLRI 01223 746720	
Project No.	PP9485757	
Project title	The role of the immune system in cardiovascular disease	
Project purpose	To understand why our immune defence system goes awry and contributes to the development and progression of these diseases, with the aim to find appropriate treatments.	

Date licence was granted	6th July 2021
Date of retrospective assessment is sent to the PPLh	02nd May 2025

The following are the triggers for licence Retrospective Assessment:
If you are completing this form because you have been asked to complete a Retrospective Assessment 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	X	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	X	The AWERB Standing Committee has asked for this Retrospective Assessment.

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	500	1st year	0	0	20	0	0	20
				2nd year	0	0	10	0	0	10
				3rd year	0	0	50	0	0	50
				4th year	0	0	0	0	0	0
				Total			80			80
2	Mice	Moderate	500	1st year	0	0	0	7	0	7
				2nd year	0	0	0	8	0	8
				3rd year	0	0	0	4	0	4
				4th year	0	0	5	17	0	22
				Total			5	36		41
3	Mice	Moderate	100	1st year	0	8	0	0	0	8
				2nd year	0	0	0	0	0	0
				3rd year	0	0	0	5	0	5
				4th year	0	0	0	0	0	0
				Total		8		5		13
4	Mice	Mild	25000	1st year	10	1359	568	5	4	1946
				2nd year	0	5032	634	9	31	5706
				3rd year	0	3875	625	13	165	4678
				4th year	0	5438	871	20	188	6517
				Total	10	15704	2698	47	388	18847
5	Mice	Moderate	7500	1st year	0	68	28	2	1	99
				2nd year	0	232	1	244	15	492
				3rd year	0	333	59	5	13	410
				4th year	0	150	74	0	7	231
				Total		783	162	251	36	1232
6	Mice	Moderate	6000	1st year	0	0	100	71	0	171
				2nd year	0	0	395	232	5	632
				3rd year	0	1	545	61	4	611
				4th year	0	60	369	93	1	523
				Total		61	1409	457	10	1937
7	Mice	Severe	1200	1st year	0	0	4	10	9	23
				2nd year	0	0	24	99	37	160
				3rd year	33	9	14	120	21	197
				4th year	0	8	26	114	5	153
				Total	33	17	68	343	72	533
8	Mice	Severe	3500	1st year	0	0	0	0	0	0
				2nd year	0	0	1	4	50	55

				3rd year	0	2	26	52	28	108
				4th year	0	0	65	38	85	188
				Total		2	92	94	163	351
9	Mice	Moderate	500	1st year	0	0	0	0	0	0
				2nd year	0	0	0	0	0	0
				3rd year	0	0	0	0	0	0
				4th year	0	0	0	0	0	0
				Total	43	16575	4514	1233	669	23034

*If actual severity data has not been formally assigned at the point the Retrospective Assessment 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 than expected	2% severe severity. 4 mice should have been assigned to protocol 7 because experiments have been carried on them, and 18 mice should have been assigned to protocol 5 (fibrillin mice). For these mice, it's an incorrect assignment to Protocol 4. These mice were found dead and automatically assigned a severe severity. The vast majority (94%) of the remaining mice were found dead/missing before weaning and were assigned a severe severity.
5	Higher than expected	3% severe severity. 18 mice were found dead (after weaning) and automatically assigned a severe severity. 8 out of those 18 mice were fibrillin homozygote mice known to potentially show severe phenotypes. These mice should have been assigned earlier to Protocol 8. The remaining 18 mice were found dead/missing before weaning and were automatically assigned a severe severity.
6	Higher than expected	The severity category on Protocol 6 is Moderate. We found that 0.5% of the mice under this protocol actually had severe severity. These mice looked either "moribund", with hunched posture or grimaced and were culled.
7	Lower than expected	The severe protocols 7 and 8 were used much less than initially planned. In protocol 7 we expected up to 30% of mice to reach a severe outcome. However, this was actually seen in 13.5% of the mice. This is due to improved experience with the model and very close monitoring.
8	Lower than expected	The severe protocols 7 and 8 were used much less than initially planned. In protocol 8 we expected up to 80% of mice to die from aneurysm rupture. However, this was actually seen in 46.4% of the mice. This is due to improved experience with the model and very close monitoring.

Actual harm was therefore less that projected incidence of harm.

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).

14-03-2022: female mouse (IL-33KO) was exhibiting moderately visible "1" signs on the NC3R mouse grimace scale and being unable to bare weight on her front paws. She had a scabby tail and flaky feet. SC18 filed on 24/03/2022

22/06/2023: One male C57/B6 mouse found dead in the morning with no obvious cause of death at day 3 after platelets deplete, which is treating i.v. anti-CD42b antibody under study plan SP132659. SC18 filed on 26/06/2023

05th February 2025: One mouse was found dead before experimental product was administered. One mouse showed spinal deformity or mass on rump, and was immediately culled after NACWOs advice. Three other mice showed signs of hunching and piloerection on 4th of February. Necropsy was undertaken. No obvious cause of symptoms. SC18 filed on 12th of February 2025. Updated on 21st May 2025 after the histological results. The symptoms may have been the result of intramural aortic haemorrhage.

30/06/2025: Exceeded the maximum number of animals allowed on the protocol. SC18 filed 08/07/2025

In Protocol 4: 4 mice should have been assigned to protocol 7 because experiments have been carried on them, and 18 mice should have been assigned to protocol 5 (fibrillin mice). For these mice, it's an incorrect assignment to Protocol 4. These mice were found dead and automatically assigned a severe severity. The vast majority (94%) of the remaining mice were found dead/missing before weaning and were assigned a severe severity.

In Protocol 5: 18 mice were found dead (after weaning) and automatically assigned a severe severity. 8 out of those 18 mice were fibrillin homozygote mice known to potentially show severe phenotypes. These mice should have been assigned earlier to Protocol 8. The remaining 18 mice were found dead/missing before weaning and were automatically assigned a severe severity.

In Protocol 6: These 10 mice looked either "moribund", with hunched posture or grimaced and were culled.

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
6	20 mice (1.03%): Only one mouse was assigned a severe severity (seen extremely moribund and culled). The other severity categories were: sub-threshold (2 mice), mild (8 mice), moderate (9). The mice were culled for various reasons: skin ulceration, piloerection, hunched posture, and weight loss.
7	1 mouse (0.19%)
8	7 mice (2.28%)

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

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

Protocol No.	If your numbers were less that estimated please explain why.
1	Not much need for superovulating mice
2	No new lines made by us so embryo recipients not used much
3	No new lines made by us so no need for vasectomised males

4	Heavy use of this protocol given the need to maintain colonies that had to bred as heterozygous and had to be backcrossed on a regular basis
6, 7 & 8	Less mice than initially planned were used under these protocols. The reasons are multiple; 1) initial overestimation of the number of animals needed for experiments; 2) less funding secured (than initially expected) to perform the experiments; 3) more in vitro and in silico computational experiments streamlining in vivo experiments (some in vivo experiments have been abandoned and re-designed based on in vitro and in silico data).

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

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

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

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

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

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

Circumstances and nature: Culled surplus, unwanted genotype and end of breeding and maintenance All the wastage we have is under the breeding protocols 4 and 5 as we only move mice to the other protocols when they are ready to use. We have a lot of strains that are crossed together. Some with two genes and some with three. This leads to a lot of wastage in breeding these strains up. Also, as explained above, we have a lot of strains where we use het x het breeders so the experiments can be wildtype vs ko. This again leads to a lot of wastage with heterozygous mice not being used. 50% of mice culled as wrong genotype.
Estimated number or percentage of animals wasted and if appropriate provide this information by protocol: Protocol 3 culled surplus and unwanted genotype – 7 breeding and maintenance – 1 Protocol 4 culled surplus and unwanted genotype – 11376 breeding and maintenance – 2970 Protocol 5 culled surplus and unwanted genotype – 526

breeding and maintenance – 319

Protocol 6
culled surplus and unwanted genotype – 24
breeding and maintenance – 12

Protocol 7
culled surplus and unwanted genotype – 17
breeding and maintenance – 0

Protocol 8
culled surplus and unwanted genotype – 2
breeding and maintenance – 0

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

Breeding in trios using targeted breeding schemes, genotyping pups at the earliest to identify pups with desired genetic alteration. Cryopreservation also done to maintain and preserve certain genetic background for future use.

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

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

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

Our strains on the MCMS database are accessible to other users in the University.
Other researchers would be allowed to use our strains once we have given them our consent.

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

Database is not available to users outside the University

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

Please explain/provide examples.

Not single-housing mice unless necessary

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

Species	Source	Approx. percentage
Mouse	In house	80%
Mouse	Charles River/Jax	20%

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

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

Protocol No.	Original estimated Number	Amended estimated number
4	25000	29000

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

Exceeded the maximum number of animals (25,000) allowed on the PPL. The number of mice used under this PPL was 25014 on 30 June 2025.
An SC18 report has been submitted on 30 June 2025.
At the date of 10 July 2025, there were 881 mice under Protocol 4. Assuming 3 to 4 breeding periods, we estimate that approximately 4,000 additional mice will be required under Protocol 4 until the expiry date of the PPL on 06 July 2026.
We needed this amendment to continue with our (funded) studies on the immune mechanisms of cardiovascular disease.

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

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

Protocol No.	Original severity category	Amended severity category

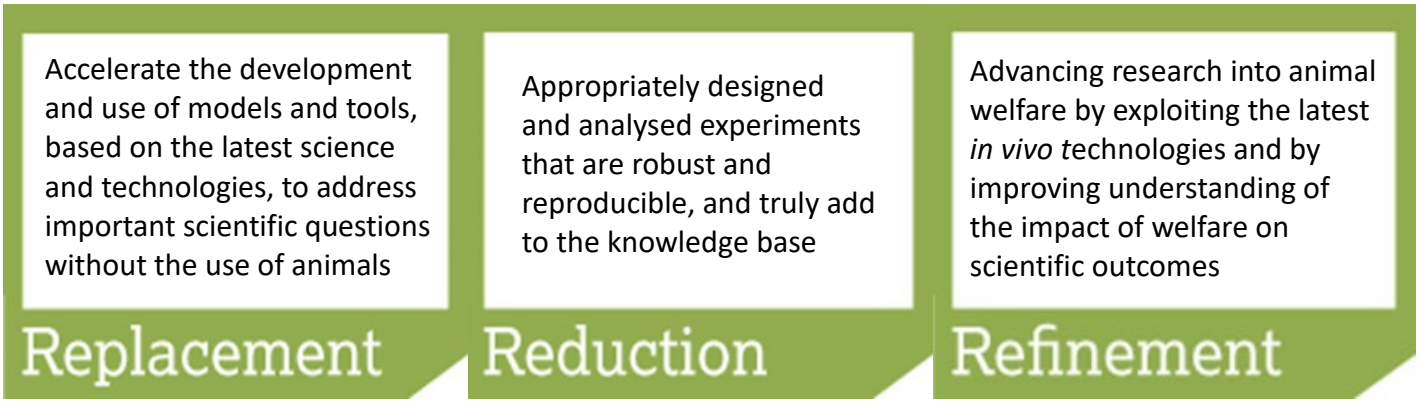
If you have increased or decreased the severity category of any of your protocols why was this necessary?

3. **3Rs:**

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

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

Definitions



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

Please explain.

More in vitro and in silico computational experiments.

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

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

We needed less mice than expected for the experimental protocols. The reasons are multiple; 1) initial overestimation of the number of animals needed for experiments; 2) less funding secured (than initially expected) to perform the experiments; 3) more in vitro and in silico computational experiments streamlining in vivo experiments (some in vivo experiments have been abandoned and re-designed based on in vitro and in silico data).

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

Please explain:

No. Our mice are accessible to other users. However, we have not had any request for tissue sharing.

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.

Techniques used:
Irradiation and bone marrow transplantation
Aneurysm development: topical elastase application on the aorta during surgery, or subcutaneous minipumps, or BAPN in drinking water.
Coronary artery ligation
Non-invasive blood pressure measurement
Vascular and cardiac ultrasound
IV and IP injections or sampling
Oral gavage
Tracheal intubation

GA animals used.
OT-II; GFP; LDLr; ApoE; AID-cre-ERT2/RosaYFP; AIDcre-ERT2/RosaYFP/ldlr; MB1Cre-; muMT; SMMHCCre/EYFP; CCR7 KO; CXCR5; Gpr183KO; RBP J fl/fl /MB1-Cre; IL-1b/MFGE8; IL-7cre; TACI/RAG2; MARK4; Balb/c; Balb/C Thy1.1; Spic-GFP; IL33KO; ST2KO; IL33/ST2DKO; Il6raTg; IL6raTg/LDLrKO; Fbn1 (fibrillin 1) Cys1039Gly; Fbn1 (fibrillin 1) Cys1039Gly/LDLRKO

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

Overall, these techniques have been very successful.
My team is experienced in the techniques listed above. We have not encountered any major technical issues, expect occasionally (for example some failed tracheal intubation attempts). We introduced a refinement method to avoid animal suffering and death in case of 2 consecutive failed attempts at inserting a ventilation tube.

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

No notable limitations

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

YES **NO**

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

Techniques have already been refined over the previous years.
During this PPL, we introduced a refinement method to avoid animal suffering and death in case of 2 consecutive failed attempts at inserting a ventilation tube (see amendment January 2024).

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

YES **NO**

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?

Daily monitoring of weight and appearance for all the mice.
Grimace scales (doi:10.1038/nmeth.1455) to monitor pain severity if necessary.
M-CASS scoring in case of LPS injection.

Protocols 7 and 8 are of severe category and require close monitoring of animals for signs of distress. In protocol 7, animals are monitored at least every day and up to 28 days to detect any sign of imminent cardiac failure (restrain of movement, hunching, breathless at rest). In protocol 8, animals are monitored at least twice each day, and in some cases every 2 hours during the next working day after injection of the anti-TGFbeta antibody to detect signs of distress suggestive of aneurysm rupture, before rupture actually occurs. We also perform serial non-invasive imaging on those mice (ultrasound) to try to detect imminent signs of aortic rupture.

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

Please explain.

We did not have to change any major endpoint, whether scientific or humane (in particular humane endpoints related to signs of imminent aortic dissection, or weight loss).

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.

Perfusion fixation
Exsanguination
These methods have been used very rarely

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

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

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

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

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

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

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

4. Scientific Progress and Benefit Achievements:

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

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

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

Objective 1: Understand the role of specific immune cell subsets (innate lymphoid cells type 2, regulatory T cells and germinal centre B cells) in the pathophysiology of atherosclerosis: We have identified distinct roles for these immune cells in either promoting or protecting against atherosclerosis (see refs 8, 14, 19, 20, 21, 22, 23, and 28). A highlight of these studies is the identification of a major role for arterial resident macrophages in the protection against the initiation of atherosclerosis. The work has been published in *Nature*.

Objective 2: Understand the role of specific immune cell subsets (innate lymphoid cells type 2, regulatory T cells and B cells) in the pathophysiology of ischaemic injury: We have identified distinct roles for these immune cells in cardiovascular remodelling post-ischaemic injury (see refs 1, 4, 10 and 13). A highlight of these studies is the identification of a major role for a new subset of B lymphocytes (marginal zone B cells) in cardiac remodelling post-MI. This has led to a translational project, developing a new drug to target this pathway.

Objective 3: Understand how alterations in vascular cells (smooth muscle cells) affect the inflammatory response and how this impacts on the development and rupture of vascular aneurysm: We identified important biological mechanisms controlling the interactions between immune and stromal cells and their roles in the development and complications of aortic aneurysm (see refs 2, 6, 11, 12, 18 and 31). We also contributed an important report on Recommendations for Design, Execution, and Reporting of Studies on Experimental Thoracic Aortopathy in Preclinical Models (see ref 30 below).

Objective 4: Understand how alterations in cardiac cells (cardiomyocytes, endothelial cells or fibroblasts) affect the inflammatory response and how this impacts on the development and progression of heart failure: We have identified an important mechanism of cardiac cachexia (see ref 27) and a new mechanism of regulation of cardiac contractility (see ref 7).

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.).

N/A

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 benefits include the design and development of relevant rodent models of cardiovascular disease that can be used by other researchers worldwide (for example we have developed a new model of cardiac cachexia); better understanding of the pathophysiology of atherosclerosis, myocardial infarction and vascular aneurysm; identification of new targets for disease limitation.

Indirect long-term benefit: better understanding of the role of specific immune cell subsets in disease state; clinical testing of novel immune-based strategies to limit the deleterious consequences of atherosclerosis, myocardial infarction and vascular aneurysm. Our pre-clinical studies formed the basis for several early phase clinical trials in patients with cardiovascular disease.

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?

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

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

We have generated better animal models of human cardiovascular diseases.
We have identified new immune mechanisms of cardiovascular disease, which led us to conduct early phase clinical trials in patients with cardiovascular disease. The results of these trials are very encouraging. We have just completed the IVORY trial in which we showed that low-dose IL-2, which activate Tregs in patients with acute coronary syndromes, significantly reduces vascular inflammation in patients. These results confirm our previous results in mice and translate them successfully to the clinical setting.

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:

1. Melhem NJ, Chajadine M, Gomez I, Howangyin KY, Bouvet M, Knosp C, Sun Y, Rouanet M, Laurans L, Cazorla O, Lemitre M, Vilar J, **Mallat Z**, Tedgui A, Ait-Oufella H, Hulot JS, Callebert J, Launay JM, Fauconnier J, Silvestre JS, Taleb S. Endothelial Cell Indoleamine 2, 3-Dioxygenase 1 Alters Cardiac Function After Myocardial Infarction Through Kynurenine. ***Circulation***. 2021;143(6):566-580.
2. Vandestienne M, Zhang Y, Santos-Zas I, Al-Rifai R, Joffre J, Giraud A, Laurans L, Esposito B, Pinet F, Bruneval P, Raffort J, Lareyre F, Vilar J, Boufenzer A, Guyonnet L, Guerin C, Clauser E, Silvestre JS, Lang S, Soulat-Dufour L, Tedgui A, **Mallat Z**, Taleb S, Boissonnas A, Derive M, Chinetti G, Ait-Oufella H. TREM-1 orchestrates angiotensin II-induced monocyte trafficking and promotes experimental abdominal aortic aneurysm. ***J Clin Invest***. 2021;143(6):566-580
3. **Mallat Z**. Dietary sucrose induces more atherosclerosis than dietary fat in LDLr -/- ApoB 100/100 mice: Is it independent of differences in plasma cholesterol levels? ***Atherosclerosis***. 2021; 325:117.

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Publications pending, or under review:

Presentations at scientific meetings:

2021
June 2021: European Atherosclerosis Society virtual meeting
Nov 2021: Singapore National Cardiovascular seminar
Dec 2021: Yale University seminar series
Dec 2021: NUBI University of Newcastle

2022
Jan 2022: Milner seminar series talk
May 2022: 3rd Conference on Immunometabolic Mechanisms of Atherosclerosis, Mexico
May 2022: European Atherosclerosis Society congress, Milan, Italy
June 2022: Vulnerable Plaque Meeting, Galway, Ireland
September 2022: The 4th Cardio Summit meeting, Graz, Austria

2023
EAS Congress April 2023, Mannaheim, Germany
German Cardiac Society April 2023
Gordon Conference on Atherosclerosis, 18 -23rd June, Spain
British Atherosclerosis Society Annual meeting | Thurs 7 - Fri 8 September 2023 | Churchill College, Cambridge, UK – Chairperson
The Imperial Vulnerable Plaque and patient Meeting (VPM), September 18-20, live webinar.

2024
4th Immuno-Metabolic Mechanisms of Atherosclerosis Conference, Caucun, Mexico, 4th – 7th May 2024, Invited speaker
EAS 92nd Congress 26-29th May 2024 Lyon, France, Invited speaker
British Atherosclerosis Society/ BSCR Annual meeting | June 2024 | Manchester, UK
Cardioimmunology Meeting June 25th - 28th 2024, Banz Monastery (Germany) invited to give a talk session titled “Clinical Trials”
International Vascular Biology Meeting, 2nd – 5th July, Amsterdam, The Netherlands, 2024, Invited Speaker Neuro-Immune modulation of vascular function
Nobel Symposium on Inflammation and Immunity in Atherosclerosis, October 2024, Stellenbosch, South Africa, Invited Speaker.

2025

lecture invitation: TRR259 5th International Symposium on Aortic Diseases 7th – 9th April, Bonn, Germany 2025 (5th International Symposium on "New mechanistic insights into aortic diseases: From molecular mechanisms to clinical applications) 11 – 11:30am talk 20 mins, questions 10 mins.
EAS Glasgow – 4th to 7th May 2025
GRC Atherosclerosis 22nd to 27th June
ESC Congress 31st August 2025
Munich Atherosclerosis consortium CRC1123 - October 21 and 22, 2025, Invited speaker

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

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

If you answered NO: why?

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?

Examples include press releases and interviews by several newspapers in the UK and abroad after the publication of our work (published in *Nature*) showing that early and intermittent hyperlipidaemia increases the risk of atherosclerosis.

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.

10 Nov 2021: typo corrected 4 June 2022: Administrative amendment to add CRUK as an authorised establishment under protocol 6. 14 Dec 2022: studying the impact of endotoxin on myocardial infarction January 2024: 1) generation and use of germ-free mice in experiments to assess the impact of gut microbiota on cardiovascular disease, 2) use of pregnant dams and their offspring to assess the impact of pregnancy on cardiovascular disease, 3) introducing a refinement method to avoid animal suffering and death in case of 2 consecutive failed attempts at inserting a ventilation tube. July 2025: ongoing, requesting an increase in the number of mice used under Protocol 4: Breeding and maintenance of genetically altered animals (mild).

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.

14-03-2022: female mouse (IL-33KO) was exhibiting moderately visible "1" signs on the NC3R mouse grimace scale and being unable to bare weight on her front paws. She had a scabby tail and flaky feet. SC18 filed on 24/03/2022 22/06/2023: One male C57/B6 mouse found dead in the morning with no obvious cause of death at day 3 after platelets deplete, which is treating i.v. anti-CD42b antibody under study plan SP132659. SC18 filed on 26/06/2023 05th February 2025: One mouse was found dead before experimental product was administered. One mouse showed spinal deformity or mass on rump, and was immediately culled after NACWOs advice. Three other mice showed signs of hunching and piloerection on 4th of February. Necropsy was undertaken. No obvious cause of symptoms. SC18 filed on 12th of February 2025. Updated on 21st May 2025 after the histological results. The symptoms may have been the result of intramural aortic haemorrhage. 30/06/2025: Exceeded the maximum number of animals allowed on the protocol. SC18 filed 08/07/2025

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).

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).

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.)?

6 Retrospective Assessment:

Project Licence Holder check list:

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

For UBS office use only:

	Date
1. Retrospective assessment form sent to project licence holder	02/05/2025
2. Retrospective assessment form received by the Project Support Team	
3a. Project Support Team meeting	
3b. 3Rs committee	
3c. AWERB Standing Committee	
4. Process completed	