employed, as rejuvenation therapies are anticipated to be most beneficial for human patients aged 60 and older. The relatively short natural lifespan of mice accelerates the study of the ageing process, expediting the development of therapies for human age-related diseases. Significantly, the ease with which mice can be genetically altered allows for the tracking of donor and host cells, facilitating the study of the effectiveness of blood stem cell engraftment — a key indicator for the successful outcome of bone marrow transplants.

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In skin studies, animals typically undergo a surgical procedure under general anaesthesia, involving a skin punch not exceeding 10mm in diameter. We will evaluate the benefit of rejuvenation on the recovery process. Upon completion of the protocol, mice will be humanely killed and samples collected.

For blood studies, animals will be exposed to irradiation to deplete their current blood system, followed by injection with donor blood cells, commonly administered through a tail vein. Post-transplantation, blood samples will be collected from the animals to assess their recovery and response to rejuvenation. At the end of the protocol, mice will be humanely killed and samples collected.

In studies examining safety with respect to cancer, mice may be injected either subcutaneously (under the skin) or intravenously (tail vein) with cancer cells of interest. Our interests include whether our rejuvenation methods impact the tumour burden and how the CAR T cells react with the tumour cells. Therefore, the animals will be humanely killed while the tumours are still small.

These experiments will help determine the safety and efficacy of therapies which can be progressed to help human patients.

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In most instances, animals are not expected to exhibit harmful phenotypes. Nonetheless, some animals may have an altered immune system, rendering them more susceptible to infection. Animals with modified immune status will be housed in a barrier environment, thereby minimising the likelihood of compromising their health.

Furthermore, aged mice often display grey fur and increased weight. Additionally, ageing animals may occasionally experience partial or complete blindness, primarily due to age-related vision loss and, less frequently, the development of cataracts or corneal dystrophy. They may also engage in scratching behaviour, resulting in minor superficial wounds. While the loss of vision is not anticipated to affect the mice's behaviour, if it is caused by a cloudy eye, it can lead to discomfort and, in the worst-case scenario, ulceration of the eye.

Animals that have undergone irradiation or treatment with specific drugs, such as tamoxifen, are more susceptible to weight loss. Therefore, these animals will be closely monitored and weighed regularly.

Some animals receiving subcutaneous (under the skin) injection of tumour cells may exhibit discomfort due to the tumour mass, which could impede normal movement.

Animals used in wound healing studies typically experience a rapid recovery from surgery. However, they may display signs of swelling, which typically resolves within 48 hours. The entire wound healing process naturally concludes within 14 days post-surgery without any intervention.

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Model	Severity	Percentage
Mice	Mild	60%
Mice	Moderate	40%
Mice	Severe	0%

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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The entire program's animal usage will be streamlined through meticulous planning and scheduling of breeding and experiments, ensuring that the minimum necessary number of animals is employed to address research queries and conduct unbiased studies.

Moreover, in previous studies, we have adhered to the NC3Rs guidance and utilised the experimental design tool (https://www.nc3rs.org.uk/experimentaldesign-assistant-eda; https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction). We have also considered the PREPARE guidelines, guiding our animal experiment planning to achieve meaningful results while minimising the animal count. These tools, along with insights gained from prior projects, will be instrumental in our ongoing

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use are not anticipated to exhibit adverse effects, and the procedures we perform are designed to be minimally invasive, with an emphasis on avoiding long-lasting harm.

In our wound healing experiments, we employ purpose-built skin punches designed to collect tissue quickly, creating up to two wounds per mouse. These resulting skin wounds are small, promoting rapid healing and minimising animal discomfort. We restrict the size of skin punches to a maximum of 10mm in diameter, and the procedure is exclusively performed under anaesthesia and analgesia to minimise pain for the animals. Additionally, aseptic bandaging may be applied to prevent wound deterioration while allowing the animals to move freely.

For experiments requiring the irradiation of mice to eliminate specific cells and alter blood cell composition, we adopt a strategy to mitigate suffering. This involves splitting the irradiation and co-injecting helper cells.

In the investigation of immune cell and tumour interactions, some mice will be subjected to the development of skin tumours. Close monitoring is implemented to minimise the duration of illness in these animals, and humane euthanasia is employed if the tumours exceed a size of 15mm.

dose will be administered in a split dose to mitigate the potential risk of severe tissue damage from the conditioning regimen.

In ageing studies, endpoints such as behaviour, body weight, appearance, and biochemical markers will be used to assess markers of ageing. This approach ensures that animals are not subjected to prolonged pain until death from old age. These markers will be continuously monitored to ensure the well-being of mice, and humane culling will be implemented to prevent unnecessary suffering if necessary.

We will adhere to guidelines pertaining to record-keeping, surgery, education, training, and reporting of experimental results. Additionally, we commit to following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, which encompass aspects such as study design, randomization, bias prevention, and statistical analysis of results.

For all procedures and animal care, we will strictly adhere to the guidance notes and webinars provided by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) (https://www.nc3rs.org.uk/welfare-assessment) and those from the Jackson Laboratories (https://www.jax.org/news-and-insights/jaxblog/2016/march/experimental-design-top-four-strategies-for-reproducible-mouse-research).

In the context of cancer studies, we will refer to the guidelines outlined by Workman et al. in "Guidelines for the welfare and use of animals in cancer research" (2010, Br J Cancer 102(11): 1555-1577. doi: 10.1038/sj.bjc.6605642).

For management of aged mice, we will refer to guidelines outlined by Wilkinson et al. in “Progressing the care, husbandry and management of ageing mice used in scientific studies” (2020, Sage Journals 54 (3): 225-238. doi: 10.1177/0023677219865291).

By consistently following and consulting these guidelines, we aim to contribute to a culture of transparent, efficient, useful, and reproducible research. This commitment is essential in minimising animal suffering and ensuring that any such suffering is only justified in instances where it is necessary for significant contributions to medicine and science.

We will consistently check the NC3Rs website to stay updated on the latest information and advice regarding the refinement of our procedures and the minimization of suffering. Additionally, we will refer to the RSPCA website (https://science.rspcg.ukence.rspdicine will M we mr"