NON-TECHNICAL SUMMARY

The phosphoinositide network in health and disease

- A small number of aged and young mice will receive an injection (either under the skin on the flank or into the peritoneum) of insulin to test their metabolic responsiveness
- A small number of mice will be bred to have a combination of genes that will lead to the slow development of prostate cancer and will be observed very carefully to ensure that the mouse does not experience significant symptoms, will not be allowed to progress beyond 16 months, and will be humanely culled.
- A small number of mice will be injected with tumour cells under the skin of the flank. The mice develop small tumours relatively rapidly but will be culled as soon as a reliable measurement of the rate of tumour progression can be made, much previous work shows this varies between 2 and 3 weeks depending on the tumour cell line. The well-being of the large majority of these mice is not impacted by the tumour.
- Potential medicines we use to treat a small number of mice, or further genetic modifications we introduce into mice, we expect to reduce or stop the ageing process, the emergence of metabolic disease or prostate cancer development.
- No mice will be reused in experiments.

For most of the mice, including immunodeficient strains (that is mice with a specifically weakened immune system), we do not expect any impacts or adverse effects in our high-quality specific pathogen-free (SPF, that is environment without any disease-causing microbes) animal care facility. NOD SCID Gamma (NSG) immunodeficient mice can exhibit progressive hearing loss that can be profound at three months of age.

Embryo transfer and vasectomy are surgical procedures with short term post-surgical pain. Post-surgical pain will be controlled by giving pain relief and any animal not fully recovered (eating, drinking, return to normal behaviour) within 24 hours will be euthanised.

Although ageing is a major risk factor for adverse effects, we know that the vast majority of our aged mice remain healthy throughout the duration of their lifetime. UirÃei ccḛ̄̄oc adverse effects not obserÎoung wilt-type mice utingUtiarchoc]ẙe bnormalityes amdominal ;welling move$issues trÁors and Ďres. tiny ₽₽% mice rem̆Ŏmdoe

proportion will be allowed to progress to a maximum of 14 months. In this model there is no evidence that the tumour spreads to other tissues and the large majority of animals will reach 14 months without exceeding mild signs. About 2% of mice may display moderate symptoms.

Subthreshold 75.1% (9000). Mild 23.1% (2764). Moderate 1.8% (216).

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Our published results show that the phosphoinositide network does not function the same in mouse prostate organoids as it does in vivo and further that it does not function the same in cultured cells as it does in organoids.

The numbers of mice required for the generation of modified mouse strains are based on the standard operating procedures extensive experience and literature review. The numbers of mice required for the breeding and maintenance protocols is based on estimations of mouse strain numbers, experience at sustainable colony management practice and the frequency of required genotype combinations. The numbers of mice required for individual experiments are based on power calculations and statistical modelling. We input the known statistical properties (phenotype average and variation), decide upon the minimal effect size acceptable from the experiment from a biological perspective and hence calculate the appropriate group size. The number of experiments required within each protocol is based on the assumption of all go-no go decisions being positive and successful grant funding achieved.

We use the NC3Rs Experimental Design Assistant where possible and take advice from the our organisation's statistician about the most effective ways to achieve statistical power without increasing the number of animals used. We work to minimise variation where and when possible. This includes ensuring related experiments are conducted at the same time in the day every time, so that we ensure mice are at the same point in any night/day cycles in their behaviour and metabolism.

Mouse models are advantageous for biological discovery. They are small and easy to breed, reaching

holders running the experiments, particularly with regard to monitoring and welfare.

All experiments which will integrate refinements from the NC3Rs (e.g., the ARRIVE guidelines and aim to work to the PREPARE guidelines (norecopa), the LASA aseptic guidelines, LASA Diehl guidelines on volumes and frequency limits (Diehl et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. 2001 J. Appl. Toxicol. 21, 15-23) and the most up-to-date veterinary knowledge. We work to the HO guidelines for efficient breeding. In our work with mouse models of cancer we will follow guidance on end points as described in Workman, P., Aboagye, E., Balkwill, F. et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102, 1555Ɉ1577 (2010). https://doi.org/10.1038/sj.bjc.6605642.

We will actively stay updated with our field of research through collaboration, conference attendance and reading the literature. We will take particular note of any technical advances that enable reduction, refinement or replacement in our experimental design. The local mouse facility is also a key source of knowledge, transmitting the latest information on the 3Rs to researchers. Internal protocols are shared across the organisation, enabling rapid uptake of any improvements to the method across groups.

Our NIO (Named Information Officer) is pro-active in sharing 3Rs updates on a monthly basis through a newsletter.