G. NON TECHNICAL SUMMARY (NTS)

Project title: Mouse models for tumour stem cells and anti-tumour efficacy studies
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: **YES**
(b) translational or applied research with one of the following aims:
(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: **NO**
(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants: **NO**
(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes: **NO**

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b): **NO**
(d) protection of the natural environment in the interests of the health or welfare of man or animals: **NO**
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work: **NO**
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills: **NO**
(g) forensic inquiries: **NO**

Keywords:
Cancer, Brain tumours, tumour growth and invasion

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Almost all malignant brain tumours represent unmet clinical needs and, on average, the life of a patient with a brain cancer is cut short by 20 years.

Our aim is to find novel brain tumour cancer vulnerabilities and to test novel potential therapeutic agents in a preclinical setting in the context of the complex processes underlying brain tumour growth and invasion.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?:
Current brain tumour treatments cannot provide a cure and the tumour frequently re-grow despite of DNA-damaging therapy. Therefore, therapeutic approaches that selectively target brain tumour cells more efficiently are urgently needed. However, the complex nature of brain tumours (including invasion of healthy brain tissue, differences between patients and within one and the same tumour) poses a challenge for successful treatment and almost all aggressive brain tumours remain incurable. Our research aims to provide a better understanding of brain tumour biology and we aim to determine the

preclinical effects of untested agents or agent combinations (for example chemicals) that could potentially stop the growth of the brain tumour in mice. The testing of agents that reduce tumour growth in animals is required for the ultimate goal of testing a new treatment in the clinic.

What types and approximate numbers of animals do you expect to use and over what period of time?:
We expect to use ~800 mice during the next 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?:
Mice undergoing surgery (intracranial cell implantation) show mild or no symptoms after they awake from anaesthesia. The level of severity is moderate. Intracranial tumour growth may lead to symptoms once the tumours become larger and once mice start showing signs of pain, they will be humanly killed.

Application of the 3Rs
Replacement:

Our ultimate goal is to translate some of our research findings, for example the use of a novel anti-brain tumour agent into a clinical test. This route requires pre-clinical studies using animals such as mice providing data that may highlight a so called therapeutic window (meaning that the inhibition of tumour growth outweighs the side effects). The mouse models described in this licence application are required for that purpose because the pharmacological aspects (including blood brain barrier penetration of any given agent) as well as the effects on brain tumour cell biology can be assessed in the relevant environment (brain), hence, allowing for sound analysis. There is currently no replacement for these pre-clinical brain tumour models.

Reduction:

All animal treatment experiments that cannot be based on literature (due to addressing a knowledge gap) will be based on comprehensive cell culture analysis including toxicity in cancer cells as compared with non-cancerous (control) cells. Experimental design will be informed by statistical tools that predict variability within the experiment so that the minimum number of animals can be used.

Refinement:

Orthotopic tumour models are currently the most refined method to address tumour development in the relevant organ. These cancer models are well established in mice. A publication database search of the key words "orthotopic, cancer, mouse model" retrieved 701 entries as of Nov. 23rd 2010 and 3725 entries as of March 10th 2016. This ~5-fold increase in literature describing orthotopic mouse models during the past 5 years strongly suggests that these models are critically required for (biomedical) research. To study tumour complexity and resistance to treatment it is important to measure tumour behaviour in the mouse model mimicking the situation in patient tumours. Importantly, so called xenograft models reflect hallmark features of aggressive brain cancer including extensive migration of tumour cells in the brain. These models utilize cells derived from patient tumours and as such reflect well the diversity of tumour profiles observed in different patients. Discomfort and distress of animals will be limited to unavoidable procedures required for the conduct of sound research. We will consider relevant refinement(s) of the surgical procedures and imaging procedures described in this protocol. Intracranial cell transplantations in mice will be performed under

anaesthesia and pain relief medication will be given after surgery. Animal will be monitored on a daily basis and will be culled humanly when showing adverse effects.