G. NON TECHNICAL SUMMARY (NTS)

Project title: Preclinical models of brain tumours
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:
Brain tumours, brain metastases, glioma

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
Brain tumours develop in up to 35% of all cancer patients and they are associated with short survival times of less than 1.5 years from diagnosis. Effective therapies are lacking. Our goal is to develop improved therapies for brain tumours at the pre-clinical level by addressing two of the major obstacles that hinder effective treatment:

(i) Blood vessels in the brain, which are much tighter than in other tissues, hinder the passage of systemically administered drugs from the blood into the brain tumours. Approaches for improved delivery of drugs into the brain are therefore expected to improve therapeutic effects.

(ii) Brain tumours have different characteristics than tumours in other organs and they are often resistant to standard therapies. Therefore, identification and exploitation of molecular targets specific for brain tumours are required.

In line with these unmet needs, our objectives are:

(i) to use a subpopulation of white blood cells, which can efficiently penetrate blood vessels in the brain, as Trojan horses for the delivery of drugs.

(ii) to identify molecular targets specific to brain tumours and perform their validation at the pre-clinical level, thereby enhancing the clinical translation of our findings.

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)?:
Our findings are expected to inform the development of improved clinical therapies for brain tumours, thereby benefiting cancer patients. In addition, our findings will enhance the understanding of basic biology, thereby advancing the brain tumour research field.

What types and approximate numbers of animals do you expect to use and over what period of time?:
We expect to use no more than 3600 mice within 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?:
The vast majority of mice undergoing surgery show mild or no symptoms after they awake from anaesthesia. A small proportion of mice (~0.3%) may experience stroke after the surgery and these mice are immediately culled humanely. Intracranial tumour growth leads to specific symptoms once the tumours become larger; at the experimental endpoint, the majority of mice experience mild symptoms characterized by slight under-grooming. A small proportion of mice is expected to display moderate symptoms including strong under-grooming, reduced mobility, hunched posture and potentially isolation. Only a minor percentage of mice (~0.3%) may display severe symptoms characterized by lethargy or disorientation.

Application of the 3Rs

Replacement:
The development of approaches for improved delivery of drugs to brain tumours requires a model in which the white blood cells travel from the bone marrow to the brain via blood vessels. This complex process requires a whole organism and can therefore only be recapitulated in animal models.

Cancer cells growing within their natural environment (in the brain) strongly differ from cancer cells growing in cell culture. Sole analysis of cells grown in cell culture is therefore unlikely to identify good therapeutic targets. Brain is a very complex organ that can at the present not be recapitulated ex vivo, and therefore these studies require use of animal models.

Reduction:
The number of required animals is significantly reduced by including ex vivo studies, by using bioluminescence imaging that allows longitudinal study of tumour growth without necessity for multiple terminal end points, and by adequate choice of animal models for brain tumours. Moreover, as many parameters as possible are analysed within one experiment, thus maximizing the outcome per experiment. We also use statistical approaches to calculate the minimal number of animals that will allow us to obtain significant results.

Refinement:
Mouse model is an established host model for studies on cancer progression, including brain cancer, and therefore well characterized. Sufficient literature supports the correlation of cancer biology and biology of brain disorders between mouse and human. To model brain tumours, cancer cells are implanted directly into the brain or administered into blood circulation, from where they subsequently enter the brain tissue. All surgery procedures are performed under general anaesthesia and pain

killers are administered to minimize any pain potentially resulting from the surgery. Antibiotics are administered to prevent potential infections. Improvement of surgery procedures is an important part of our refinement efforts. Over the years, we optimized surgery techniques, which resulted in significantly reduced percentage of mice experiencing stroke. Animals are closely monitored - including throughout the night whenever required - to warrant their wellbeing at all times and to ensure that experiments are terminated prior to occurrence of substantial symptoms.