G. NON TECHNICAL SUMMARY (NTS)

Project title: Mechanisms of tumour development and treatment resistance
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: YES
   (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, Chemotherapy, Treatment resistance

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
It is not known why some children develop cancer; the impact of their environment and genetic changes
play a role, but the specific timing and types of genetic alteration and environmental exposures are to
be elucidated. In the case of paediatric lymphoma, a cancer of the immune system, it remains to be
determined why some forms of lymphoma occur more in children than in adults. In this project,
Genetically Altered (GA) mice will be used to identify genetic drivers of disease: Genetic changes
detected in human tumour tissues will be engineered in mice to assess their contribution to tumour
development in vivo in established GA model systems. The effects of environmental exposures such as
viral infections will also be analysed in these model systems to determine whether these processes
force tumour development in the lymphoid system. The established GA models will then be applied to
investigations of novel therapeutic agents in order to develop less toxic treatments than the
chemotherapeutic agents currently in use. We will also apply these findings to patient derived
 xenografts – mice propagating human tumours as 'hosts' or 'avatars' of their disease thus taking into
account patient genetic variability. In doing so, we will also identify the tumour cells that are responsible
for disease relapse.

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)?:
Through the course of this project we will not only shed light on why and how children develop lymphoma and other cancers but we will also derive new therapeutic approaches. Current therapy is toxic leading to life-long health problems. Our model systems will inform on new, less toxic therapies that not only lead to disease remission and cure but also prevent relapse from occurring. Specifically, this work will be conducted applying to certain types of childhood cancer including neuroblastoma and lymphoma that carry mutations in a gene called ALK.

What types and approximate numbers of animals do you expect to use and over what period of time?:
Approximately 5000 mice will be used over the course of the 5 years of this project. This number of mice is required so that all of our experiments are conducted with sufficient replicates to provide meaningful data with statistical significance before we progress to first-in-man studies.

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 mice in this project are housed in a controlled environment in a barrier unit. They are tended to by qualified technicians who ensure their health and safety, clean housing by regular inspections and cleaning protocols. The mice are also provided with an enriched environment with stimulating toys such as tunnels and wood sticks. The large majority of work will be conducted with genetically altered mice that express genes previously shown to be prevalent in human patient cancer cells. These mice will be aged for tumour growth; once tumours have grown the mouse will be killed, tumours removed and studied ex vivo. As such, procedures conducted on the mice are minimal. All mice will be closely monitored for any clinical signs of ill health so that no more than transient harms are experienced. This will be conducted by daily monitoring of their health status and a score sheet to assess the cumulative effects of any treatment. At present, we are unable to grow these tumours in plastic dishes as they require a number of support systems that we cannot yet mimic in vitro. Sometimes, it is not possible to detect tumour growth in the mice and they may get sick very quickly once it reaches a critical mass (normally this occurs overnight). This can happen in up to 25% of our mice and in these cases, as soon as the mouse is discovered to be ill, it is killed by a Schedule 1 method. Some mice will receive injections of anti-cancer agents in an attempt to cure them of their cancer. Some of these agents may cause the animals to suffer ill health as do humans on treatment with chemotherapy but we will ensure that any clinical signs are detected and treated within 24 hours. If it is not possible to alleviate their symptoms, the affected mice will be killed by a Schedule 1 procedure. The injections themselves will give rise to minimal pain and suffering and will be transient. When we wish to grow human tumour cells in mice, we may first expose them to radiation to kill their immune system to essentially create a mouse that has no immune system. When lacking an immune system, the mice will not reject the human tumour cells. As these mice have a suppressed immune system they will be housed in isolator cages, provided with sterile food and water, and monitored closely for any signs if infection. These mice may also be exposed to chemotherapeutic agents at low doses to induce resistant tumour cells to form. All animals will be killed by a Schedule 1 method at the end of the procedures.

Application of the 3Rs
Replacement:
Cancer is a disease involving the whole body; tumour cells recruit a variety of other cells within the body to support their growth. This includes new blood vessels to support their increasing requirements for food, nutrients and oxygen as well as a stromal network to support its size, and inflammatory cells to provide growth factors and other growth-promoting signals. As such, cancer can only be fully mimicked in an experimental setting where all of these facets can be taken into account. At present, we have limited other model systems which at best can only replicate a few properties of these growth support systems. A second consideration are the effects of chemotherapeutic agents and their distribution within the body – their so-called bioavailability. Whilst some drugs may work very well in a
tissue culture dish, when applied to a whole-body scenario, they often fail due to the above-mentioned facets. Therefore, to truly mimic cancer treatment, the whole body, its response to the tumour and to chemotherapy must be evaluated. However, we maintain close attention to the scientific literature for any techniques that might improve our ability to replicate tumour growth in plastic dishes and at the same time, every time we grow a tumour in a mouse, we also attempt to refine conditions for its growth in plastic.

Reduction:
Every experiment will be conducted following consultation with a statistician or the performance of Power calculations to ensure that we use the minimal number of animals to give significant and meaningful results. We will also use existing genetically altered mice rather than generating new models where practical and monitor databases of genetically altered mice towards this aim.

Refinement:
Mouse models of cancer have been developed over many years to mimic the human disease in order to facilitate research into how and why cancer develops and therefore how best to treat it. By refining these models we have been able to further our understanding of cancer considerably – something that could not be achieved with less sentient systems. Largely, this is because cancers are not autonomous growths of cells, i.e. they cannot grow on their own without the support of the microenvironment including, for example, a blood supply, the immune system, an extracellular matrix and a variety of proteins, chemicals and growth factors. Until we have determined what all of the support systems are, we will not be able to fully model human cancer in a petri dish. As such, we continue to work with a murine model whilst also developing in vitro systems. All techniques will be conducted by experienced staff that are trained to be competent and are regularly assessed, therefore reducing any suffering to the mice. All experiments will therefore be conducted in a timely manner and when necessary with the use of anaesthesia. Mice are housed in a designated facility under sterile conditions in enriched housing environments.