Home Office

NON-TECHNICAL SUMMARY

Imaging and radiation treatment of cancer

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
    - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
    - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (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 following aims mentioned in paragraph (b)

Key words

Radiotherapy, Cancer, Radionuclide, hypoxia, Antibody

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overarching aim of this project is to improve the outcome of cancer patients by enhancing the effectiveness of radiotherapy.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Radiotherapy is a highly effective therapy used in the treatment of 50% of patients with cancer. There are several different types of radiotherapy. External beam radiotherapy is applied in a radiotherapy department using an external radiation source which is rotated around the patient producing a series of radiation beams that intersect in the cancer. This means that the cancer receives a large dose but the surrounding normal tissue - organs etc - receive a smaller dose. The greater the dose that the cancer receives, the greater the chances that it will be destroyed. However the dose of radiation that can be given is limited by damage to normal tissue.

Targeted radiotherapy (TR) which is used to treat lymphoma and prostate cancer and is being rolled out to other cancer types due to its proven effectiveness. Cancer cells have on their surface, markers that are not found or are found in far less amounts in normal cells. These markers can be targeted by highly specific drugs. These drugs only bind to their selected marker. TR involves drugs attached to radioactive atoms (radionuclides) that specifically target these markers and irradiate them at close range. This type of radiotherapy is very specific and will also destroy the secondary cancers (where the main cancer releases cells that set up many new cancers throughout the patient). However cancers contain many cells which vary in the number of markers that they have on their surface - this is called heterogeneity and diminishes the effectiveness of TR as not all parts of a cancer will receive the radiation from a TR radioactive drug.

The project will address tumour heterogeneity by altering the drug so it targets several markers and by using several different radioactive atoms that irradiate cells nearby and at a distance.

As cancers grow their blood supply does not grow with them in a well co-ordinated way so they have regions within them that do not receive much oxygen - these areas are called hypoxic. Radiotherapy is much less effective at killing cancers that have large hypoxic regions. By identifying markers on hypoxic cells we can target these with TR to boost the radiation dose to these cells.

The effectiveness of targeted radiotherapy in a patient can be measured by giving the patient a version of the TR agent with an imaging beacon (another type of radioactive atom that is far less damaging to tissues) that distributes in the patient before a therapy version. The patient is imaged and the amount of RT agent measured. This project will also design and test imaging and therapy versions of targeted radiotherapy agents.

What outputs do you think you will see at the end of this project?

The work from these projects will be published in high quality cancer and radiobiology journals.

1. Optimisation of radioactive cancer marker-targeted drugs for cancer patients receiving targeted radiotherapy (TR).

2. To demonstrate that imaging can be used to predict if a TR compound will localise in cancer tissue and be effective in a patient before a therapeutic version of the compound is administered.

Who or what will benefit from these outputs, and how?

Short term - increased knowledge in developing radiotherapy-based drugs which will be published

Long term potential benefits:

Improved response of cancer patients treated with targeted radiotherapy by optimising the radioactive drugs administered to patients that seek out and destroy their cancers: Current TR compounds have one type of radioactive atom attached and targets one type of cancer marker. Cancers are heterogenous in the expression of cancer markers between different areas in a single cancer. There are also blood flow issues affecting where the TR molecules go to within the cancer. To overcome this we are developing TR compounds that have two types of radioactive atom that will emit lethal particles both nearby and at a distance from where the TR molecules localises within the cancer. We are also targeting two cancer cell markers. Our hypothesis is that this will enable a better distribution of lethal radiation within the cancer so that all the cancer receives lethal radiation and no part of the cancer is left to regrow.

Reducing morbidity from radiation damage to normal tissues: TR molecules are damaging to normal tissue if they do not preferentially go to the cancers in the patient. However it is possible to determine where the TR compounds go by labelling them with an imaging radioactive atom (which is far less damaging) and imaging the patient and calculating how much goes to each tissue. From the image the radiation dose delivered to each organ and the tumour can be determined (this is known as dosimetry). If the dosimetry indicates that a patient the benefit/harm ratio is high the patient can be given the TR compound this time with the lethal radioactive atom attached. It is also possible to tailor the amount of TR molecule that is given to the patient to reduce normal tissue damage.

How will you look to maximise the outputs of this work?

Targeted radiotherapy (TR) uses a range of radioactive atoms which produce different forms of radiation. One of the challenges with researching and clinical delivery of TR is obtaining the radioactive atoms. This work will involve collaboration with the a nuclear institute which will optimise the delivery of several clinically useful radionuclides. Collaboration with a Government nuclear laboratory will ensure a source of 212Pb which is a very effective cancer killing radioactive atom. Ensuring a broad stakeholder audience for the developments from this project will be assured through interaction with the CRUK steering group on targeted radiotherapy.

It is envisaged that the project work described here will provide useful methodology for advancing radiotherapy. However it is possible that not all approaches will prove to be effective. These less positive outcomes will still be published.

Species and numbers of animals expected to be used

- Mice: 800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The cancer models that are required for this work are cancers growing in nude adult mice or rats. These animals are the least sentient organism in which cancers can be grown that can receive radioactive drugs and be monitorable with medical imaging modalities such as PET. The cancers (xenografts) need to be of a large enough size to contain emissions of radioactive atom which can be 1cm in distance so a 1.2cm3 (1.0cm diameter) is required.

Typically, what will be done to an animal used in your project?

Cancers will be generated in nude mice by injected cancer cells or pieces of cancer into one of the flanks of anaesthetised animals. Animals are anaesthetised which is carried out by placing them in a tank filled with an anaesthetic gas. The animals are kept anaesthetised via delivery of anaesthetic gas through a face mask. Insertion of cancer cells via a needle by insertion of a piece of tumour into a flank via a tube with an outer diameter of about 3mm. In both cases the needle is inserted just under the skin (sub-cutaneous) and only in one flank. The animal will not be aware of the procedure until regaining consciousness - generally mice do not demonstrate any awareness that the procedure has taken place - rapidly returning to feeding.

Cancer growth will be monitored by regular inspection of the cancer site. The cancer will not be allowed to get any larger than 1.2 cm^3 volume (about the size of a standard marrowfat pea). This size will not interfere with the animals movement or cause it distress. This volume is to accommodate radiation from high-energy radiation-emitting radioactive atoms which travel up to 1.0 cm distant from the radioactive atoms. It is envisaged that for most of the studies using lower energy emitting radioactive atoms (where the radiation range is low) outlined in this PPL the maximum volume will be <1cm^3 volume (about the size of a garden pea). Mice do not demonstrate any awareness of the growing cancer. During cancer growth dimension measurements are taken using calipers. When handled during these measurements the mice are curious and do not demonstrate any signs of stress. The animal is also weighed by placing on a top-tray balance.

When the cancer is a suitable size for experiments the animal might be injected with a radioactive drug that seeks out and binds to the cancer. It is unlikely that the mice will be aware that they have been

injected as these drugs do not irritate and are suspended in a a non-irritating medium that has a neutral pH. Apart from decreasing the growth rate of the cancer the substances are unlikely to have any effect on the mice. The animal may then be killed by schedule 1 under anaesthesia between 5 minutes and several days after injection. Alternatively the effect of the radioactive drug on the growth of the cancer may be monitored over a period of several weeks.

For some experiments the animals may be imaged after injection of a radioactive substance. Imaging involves anaesthetising the animal as described above. The mouse in then placed in an animal PET scanner. During imaging under anaesthesia the animal will be placed on a heating pad to ensure the animals is kept warm. It is likely that mouse core temperature will be monitored by a rectal thermometer. At the end of the imaging session the animal is either euthanised by a schedule 1 method or allowed to recover. Some animals are scanned on two occasions with at least a 4h gap between during which time the animal will have fully recovered and be exhibiting normal behaviour.

If animals do not recover consciousness within 1h they are euthanised by a schedule 1 method.

What are the expected impacts and/or adverse effects for the animals during your project?

The mice develop tumours which are unlikely to spread beyond the site of injection. Mice will be closely monitored and scoring system developed and humane end points identified and updated as required.

For some of the studies we will require a large blood sample which can most humanely be acquired by cardiac puncture under terminal anaesthesia.

If any of the procedures result in pain as evidenced by apparent facial expression (Facial expressions of pain) analgesia may be given as a first line but if this is insufficient to alleviate pain the animal would be euthanised by a schedule 1 method.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The level of severity is expected to be moderate or more commonly mild for any of the procedures detailed in this project.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Targeted radiotherapy uses radioactive atoms which emit radiation of different energies. Radioactive atoms that produce low energy radiation kill nearby cells. Radioactive atoms producing high energy radiation travels further and kills cells at a distance. One part of this project is to overcome the problem of heterogeneity in the distribution of cell kill within the tumour by combining different energy atoms to kill cells close by and at a distance. The distance that we need to observe cell binding and cell kill is several mm. We can only demonstrate the benefit of these systems in 3D using cancers growing in living organisms.

This project will produce optimised targeted radiotherapy drugs which will be aimed at clinical translation. Before any radiolabelled drug is administered to a patient it must be tested in animals and organ dosimetry determined - (where the TR compounds go and how much radiation they carry into organs) by labelling them with an imaging radioactive atom (which is far less damaging) and imaging the patient and calculating how much goes to each tissue.

Which non-animal alternatives did you consider for use in this project?

The only alternatives are tumour spheroids which are 3D models of cancer grown in vitro.

Why were they not suitable?

Tumour spheroids lack a vascular system so only grow to a few hundred micro-metres in size which are far too small for cancer heterogeneity studies.

Exploring imaging methods to characterise tumours requires an in vivo tumour model.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Radiation-sensitive cancer cells will be used to derive the xenografts to achieve the greatest effect of radiation on tumour growth. Inbred animals of similar age will be used for each study to reduce variation.

The basis of most of the work in this PPL is developing radiopharmaceuticals that can be used in man. To achieve clinical translation these compounds must be preclinically testing in animal tumour models and bio-distribution determined. The uptake and tumour distribution of radiopharmaceuticals is also dependent on a blood supply.

Similar work in the literature (human cancer cell derived xenografts grown in nude/SCID mice, receiving targeted radiotherapy) in the literature typically use 5-7 mice in each treatment group in order

to achieve mean differences at 5% significance levels. However we will be comparing the efficacy of tumour growth inhibition by different radionuclides so the differences in growth rate are likely to be smaller so we may require larger treatment groups. We have sought advice from a statistician who has recommended a pilot study to assess the magnitude of cancer growth inhibition is achieved by the radioactive drugs to determine treatment group size.

PET is very sensitive and its quantitative accuracy is highly dependent on minimal subject motion which is best achieved through anaesthesia. Image degradation through movement would inevitably lead to the requirement of more animals per time point negating the benefit of reduction in animal numbers through serial imaging.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Pilot experiments will be used to check that the transplanted cells produce regular sized cancers suitable for autoradiography and that the radiation dose administered during treatment, determined from in vitro experiments and the literature, is sufficient to produce appreciable cancer growth inhibition compared with untreated tumours. We will seek advice from the University's biostatisticians regarding actual sample sizes required informed from pilot data.

Cell lines are authenticated and regularly tested for mycoplasma contamination by the University's core facilities.

(Reference: Workman et al Guidelines for the welfare and use of animals in cancer research. Br. J. cancer 2010 102:1555-1577)

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Mice used in these studies are inbred which will reduce genetic variability between the mice.

As described above we will use pilot studies to determine the growth inhibitory effect of targeted radiotherapy on xenografts to inform on the actual number of mice needed in the control and treatment groups.

Where possible experiments will be designed so that multiple treatment groups can be compared with a single control group.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The procedures carried out in this study are the induction of carcinogenesis by subcutaneous injection of cancer cells into the flanks of nude mice followed some days later by injection of a radioactive agent that targets the cancer cells. These tumours do not usually spread. Tumour growth of sub-cutaneous tumours does not result in constriction of blood vessels or organs.

Why can't you use animals that are less sentient?

Rodents are the animal of lowest sentience that can support the growth of xenografts and that can receive radiotherapy, hyperthermia and radioactive drugs. Nude mice are genetically altered so that they can be hosts to tumour derived from human cancers.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The generation of cancers in nude mice under anaesthetic will be painless. Once recovered from anaesthesia (within 1h) the mice feed and behave normally and do not display signs of discomfort. As the cancers progress the mice will be carefully monitored for behaviour suggesting discomfort. Generally the cancer enlarges with no apparent evidence of discomfort. These types of cancers (xenografts) do not usually spread beyond where the cancer cells were inserted. Occasionally (<10%) animals bearing xenografts display weight loss. Animals are weighed 2 times per week from the time the cancer cells were implanted. Once palpable the cancers are measured twice per week. From 0.75cm3 in volume (size of a garden pea) the tumours are measured daily and the animal weighed. Weight will be monitored and the mice will undergo euthanasia if their weight loss exceeds 15% of body weight from start of treatment (after subtracting an estimate of cancer weight).

PET is very sensitive and its quantitative accuracy is highly dependent on minimal subject motion which is best achieved through anaesthesia. Image degradation through movement would inevitably lead to the requirement of more animals per time point negating the benefit of reduction in animal numbers through serial imaging.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

1) Workman et al Guidelines for the welfare of animals in cancer research. Br. J. Cancer 2010 102 1555-1577

An updated version of this paper is in progress. Recommendations in the revised version on publication will be followed.

2) NC3Rs website

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

NC3Rs website to follow any developments in cancer models. If a suitable cancer model becomes available which enables 3D tumours to be generated that are several mm in diameter in vitro we will compare results from dose-distribution experiments with that from xenografts in a cell line to determine if some of the work can be carried out using the model.