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NON-TECHNICAL SUMMARY

Novel methods for cell type-specific drug delivery

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Drug delivery, Cancer, Targeted therapy

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 aim of this project is to identify new ligands (molecules that bind specifically to other molecules) that, when injected into animals, will accumulate at specific cell types and organs, with the view that in the future the ligands will be coupled to therapeutics for targeted drug delivery in humans.

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?

Targeted drug delivery is a major unsolved problem in biomedicine. Most drugs, when injected into a patient, will distribute non-specifically throughput the body, accumulating in the liver or filtered out by the kidneys. For many diseases, this results in a subtherapeutic (ie. ineffective) local drug concentration at the organ of interest, as well as toxic off-target effects due to drug accumulation in non-target organs (eg. liver toxicity). To address this problem, we must identify novel binders that display cell or organ-specific binding and accumulation. With these tools, we will be able to better treat diseases such as cancer (eg. targeted delivery of chemotherapies), because effective targeted drug therapy will reduce the likeliood of severe side effects in patients when used in the clinic.

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

The main outputs to this project will be ligands that enable drug delivery to specific organs or cell types, with improved efficiency and specificity compared to existing modalities. We envision that our ligands will be applicable to deliver a wide range of different drugs, including chemotherapy for use in cancer therapy, as well as different classes of genetic medicines. The details of these experiments will result in peer-reviewed publications and patents. We will also present our results in local and international conferences.

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

The broader academic scientific community will immediately benefit when we disseminate our results (within 3 years). On a longer time scale (5-10 years), we aim to translate results obtained in our animal studies into novel drug candidates that will begin clinical trials; this will directly benefit patients by increasing patient survival through increased drug efficacy, or by decreasing side effects of existing drugs through reduced drug accumulation in off-target tissues.

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

We aim to publish our findings in peer-reviewed journals, as well as present our results in local and international conferences, both within academic and industrial settings. We also aim to maximise our

outputs by working with different academic groups and allowing dissemination of our findings through collaborations.

Species and numbers of animals expected to be used

- Mice: 1500

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.

We are broadly interested in targeted drug delivery to a wide range of organs, in individuals that are healthy as well as tumour-bearing. Consequently, we will use wild-type mice, as well as a subcutaneous tumour mouse model. We will use mouse models because cell lines, patient samples, and other complex cell culturing techniques (eg. organoids, organ-on-chip) do not accurately recapitulate the complex interactions between different organs in a live animal, as well as certain properties in the blood of live animals which affect how quickly drugs are degraded when injected, that are particularly important when characterising drug biodistribution and half-life. We will only use adult mice, as this programme is not focused on how neonatal, juvenile or aged phenotypes affect drug biodistribution.

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

Our experiments will involve both healthy and tumour-carrying mice. The former will simply be obtained through breeding of "wild-type" mouse colonies. For the latter, our experiments will begin with tumour induction. This is achieved by engrafting animals with tumour cells, underneath the skin ("subcutaneous tumour"). Tumour development will be assessed by calliper measurements (tumour size), imaging, and blood sampling.

Once healthy or tumour-carrying mice are obtained, delivery modality candidates (sometimes linked to relevant drugs to test their delivery) will be administered, through injection into the bloodstream ("intravenous injection"), into the abdomen ("intraperitoneal injection"), or directly into the tumour ("intratumoural injection"). At a defined time point (generally after several hours, but could also be across several days), animals will either be imaged (for appropriately labelled candidates) to assess the biodistribution of the injected drug, or they will be killed humanely to allow biodistribution across multiple organs to be assessed by extraction from organs. Regardless of experimental workflow, all animals will be killed humanely at the end of each experiment.

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

General signs of distress or ill health will be carefully assessed throughout each experiment.

Tumour induction may cause mild pain and discomfort, though we have found that tumour growth is generally well tolerated. Potential adverse effects include weight loss, hunching, tremors and altered breathing, as well as general behavioural patterns associated with distress such as excessive grooming. However, these are very rare. We will closely monitor tumour-bearing animals, and any mouse displaying any such signs of discomfort will be humanely killed.

Administration of our drug candidates may cause very temporary discomfort associated with any injection. Beyond these effects at the site of injection, from our experience we do not anticipate any other side effects caused by our drug candidates. Nonetheless, any animal displaying sustained signs of discomfort after injection will be humanely killed.

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)?

We expect 10% of animals to experience mild severity, and 90% of animals to experience moderate severity.

For healthy mice used in our experiments, we expect 80% of animals to experience moderate severity, due to the use of anaesthesia for imaging. This will be time-limited suffering (1-2 hours). The other 20% are expected to experience no more than mild suffering.

Tumour-bearing mice are expected to experience no more than moderate suffering, due to the injection of tumour cells, as well as some pain and discomfort caused by the resulting tumour mass. In our experiments, animal suffering will be limited because we are not interested in late-stage disease processes; consequently, tumours will not be allowed to progress into late-stage growth. Unlikely (<5% animals) adverse effects for tumour growth include weight loss, hunching and tremors, but in our experience we have not come across these effects. Animals experiencing these adverse effects will be killed humanely.

We expect to use equal numbers of healthy and tumour-bearing animals, thereby arriving at 10% of mild and 90% moderate severity levels, respectively.

What will happen to animals used in 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?

The aim of our project is to accurately characterise the distribution of different candidates for drug delivery when injected into live animals (known as drug biodistribution). While we use other non-animal models in the laboratory to assess distribution and uptake of drugs across different cell types, ultimately these poorly recapitulate key aspects of drug distribution and accumulation inside patients. This is largely because (1) different organ systems within the body interact with each other in complex ways that can result in unpredictable patterns in drug biodistribution, and (2) properties of the blood within live animals often lead to decreased stability of a drug, which can only be accurately assessed in live animals. Therefore, in order to accurately assess the efficacy of a given candidate for drug delivery, live animals must be used.

Moreover, we have searched through existing publications and pre-prints (and will continue to do so during the course of the project) to ensure that we are not duplicating experiments already performed elsewhere, including the SyRF platform to access meta-analysis of existing animal work.

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

We have used, and will continue to use, alternatives in assessing the potential of different candidate modalities for targeted drug delivery. These include healthy and tumour-derived human cell lines, cells isolated from healthy patient blood samples, as well as cancer tissues and blood from cancer patients. In order to preliminarily assess biodistribution across different cell types without using live animals, we often co-culture cells from different organs to assess differential uptake of drug candidates across different cell types. We only carry out animal work for a given candidate if we find promising results in prior, non-animal studies.

We have also considered the use of organoid and organ-on-chip systems, as well as non-protected species (eg. chick embryos), but unfortunately these are poor models for biodistribution in humans. Consequently, we need to use mice because they are well characterised to have physiology that is similar enough to humans to yield meaningful experimental results.

Why were they not suitable?

Biodistribution is a complex, emergent property that arises from the interactions of different organ systems within a living patient or animal. Non animal models, including ones we use (such as human cell lines and patient derived samples), as well as other systems (including organoid and organ-on-chip models) are unable to accurately recapitulate and mimic drug biodistribution in patients. Chick embryos have also recently emerged as a powerful model for tumour development, but because they are a closed system, they are not suitable to evaluate drug half-life and biodistribution (eg. there is no excretion of the drug, so there would be prolonged exposure of a given dose compared to in human patients).

Using live mice, we can accurately assess drug serum half-life, unwanted accumulation in off-target tissues, as well as the effects of using different delivery schedules, as well as the age and sex of the animal. Moreover, non-protected species, including fruit flies or nematodes, are insufficient for our purposes because, unlike mice, their physiology and anatomy are too different from that of humans to be of predictive value. For these reasons, we argue that it is essential for our work to be conducted in mice.

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?

Our estimated number of animals is calculated based on our planned research activity, as well as past studies assessing biodistribution of related drug delivery modalities.

In particular, we plan to inject fluorescently labelled delivery candidates into animals, and assess their biodistribution through live animal imaging. Past studies have shown that group sizes of 6-8 animals (biological replicates) are required to detect biologically relevant differences in such experiments. In these experiments, control delivery candidates (eg. sequence scrambled controls) will be used as a baseline for comparison. In each experiment, we will likely test at least 5 candidates alongside controls, leading to 36-48 animals being used. We plan to perform 6-7 experiments of this nature every year.

This amounts to 300 animals used per year across a 5 year period. We envision a roughly equal split of experiments looking at healthy animals vs tumour-bearing animals. This amounts to 750 animals used in protocol 1, and 750 animals used in tumour induction protocol 2.

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

We will use the NC3Rs' experimental design guidance and assistant (EDA) to design our experiments in order to identify nuisance variables (eg. age of animals, time of day of drug administration etc), and eliminate associated experimental noise. This will allow us to achieve greater statistical power with fewer animals. By consulting published studies on evaluating drug delivery candidates of a similar nature, we conclude that 6-8 mice per experiment will achieve sufficient power in our statistical analyses.

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

Where possible, we will obtain transgenic mouse strains from collaborators or commercial providers instead of producing new strains ourselves. Relatedly, all breeding under this license will be managed by experienced technicians to ensure efficient breeding.

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.

Mouse models

Mouse tumour models will be used, because mice are the most well-characterised, and lowest form of mammal that is relevant for modeling human cancers. In our previous work, we found that our most promising drug delivery candidates show specificity to tumour cells from many different organ types when tested in vitro, and consequently in this project we will test whether this translates to in vivo biodistribution. These tumour mouse models will enable us to determine whether our candidates are capable of specifically delivering drugs to tumour cells, while sparing neighbouring healthy cells and in other organs of the same animal. Moreover, we will perform experiments on animals at a relatively early time point after tumour induction, allowing us to minimise discomfort in animals due to tumour burden. We will carefully characterise tumour burden (eg. using bioluminescence imaging and calliper measurements) in order to perform experiments as early as possible, before animals experience significant pain or suffering. Moreover, we will carefully monitor the health and behaviour of all animals. If necessary, animals will be humanely killed to avoid further suffering.

Methods

Throughout the project, we will use methods that will minimise pain, suffering and distress in all animals. We will administer individual drug candidates or large pools of candidates through tail vein injection, which causes suffering of a mild severity. We will follow best practices to reduce harm to animals (eg. only use single-use needles to avoid pain from using dulled needles). Our work will be carried out by staff members that are fully trained, ensuring that the highest level of animal handling.

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

Mice are the most well validated mammal for cancer models, and are highly relevant in biomedical research due to the similar physiology between mouse and man. Consequently, relevant mouse disease models will be able to recapitulate key aspects of drug biodistribution, enabling accurate characterisation of specific drug delivery candidates. Unfortunately, such experiments are simply not possible in less sentient animals, because their physiology is not similar enough to that of humans. Relatedly, embryos or very young animals are also insufficient for our purposes, because their organ and circulatory systems are not well developed enough to fully recapitulate drug biodistribution patterns in human adults. A notable exception is the recent development of chick embryos to study tumour development, but because of the closed nature of this system, drug biodistribution and half life cannot be accurately measured.

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

Throughout the work described in this license, we will continue keeping up to date with relevant literature and NC3R guidelines to ensure that our procedures are of the highest standards. Where possible we will supply mice with environmental enrichment (e.g. tubes, igloos, wooden logs or nestlets). We will also employ refined animal handling techniques, e.g. a tunnel or cupped hands. Moreover, we will closely monitor animals for signs of pain and suffering.

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

We will adhere to the NC3Rs ARRIVE guidelines and the PREPARE guidelines, as well as other published guidelines (eg. https://doi.org/10.1258/0023677011911345).

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

We plan to stay informed about advances in the 3Rs by regularly checking available information on the NC3Rs website. We are also subscribed to the NC3Rs newsletter, and we plan to attend regular local 3Rs symposia.