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G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Zebrafish models for investigating cancer formation and progression, immune responses and immunotherapy
Key Words	melanoma, zebrafish, immunity, CART
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(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.

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

1) To learn more about melanoma, including how it escapes destruction by the immune system. Melanoma is a cancer of pigment producing cells known as melanocytes that mainly reside in the skin. Approximately 12,500 new diagnoses are made each year in the UK. Around 20% of melanoma patients currently die from their disease. Exposure to ultraviolet waves from the sun results in mutations in melanocytes that consequently grow out of control. Ordinarily, the immune system is equipped to detect and remove abnormal cells but for a number of reasons this process is not always 100% effective and cancer can progress. Modern (immuno)therapies seek to restore immune responses that once more destroy cancer cells. While very promising (a significant fraction of patients have been cured), they do not always succeed: some tumours fail to respond while others stop responding.
2) To evaluate new treatments including establishing whether zebrafish can facilitate the discovery and development of chimeric antigen receptor T cell (CART) therapy. CART therapy is a new form of immunotherapy that has proved effective in treating blood cancer but not yet solid cancers like melanoma. In CART therapy, immune cells known as T cells are taken out of the patient and genetically modified to enhance their ability to detect cancer cells. They are then reintroduced into the patient. Current obstacles with CART therapy is lack of potency and equally problematic the possibility of serious autoimmune-style toxicity.
3) To learn more about the immune system of zebrafish. Our knowledge of immunity in fish trails far behind that in rodents and humans. Among fish species, zebrafish has not been intensively studied as it is of no commercial value. But owing to the potential of zebrafish models for generating insight into human disease mechanisms, and also embracing technological advances that can rapidly generate data, it is now worth addressing that gap.

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)?

1) The research will reveal the disease mechanisms which result in the formation, maintenance and progression of melanoma.

2) The research will identify and validate novel biological targets for drugs that can treat melanoma.

3) The research will expand our understanding of the function of the immune system in zebrafish, and indicate whether it is a suitable model for research into human disease. It could also benefit the aquaculture industry that is trying to improve disease management in fish stocks through developing vaccines, which requires knowledge of immune system function.

4) If we are successful in advancing basic understanding of the function of the immune system in zebrafish, subsequent research could uncover what limits host immune responses to cancer and how to improve immunotherapy of cancer.

What types and approximate numbers of animals do you expect to use and over what period of time?

22,500 zebrafish over 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?

Genetic modification of these animals could result in genetic disease or cancer, causing moderate suffering. The implantation of cancer cells and exposure to experimental treatments with unanticipated toxicity could also cause moderate suffering. Zebrafish will also be treated with factors that stimulate immune cells which is assumed to elicit only mild irritation. During investigations, they may be rendered temporarily unconscious using anaesthetic or in limited circumstances by inducing hypothermia in order to image animals, for which they need to be still. At the end of study, the animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Exploratory studies, where possible, are first performed in a test tube or in zebrafish embryos. Data is also generated from human tumours. However, the involvement of multiple cell types in the process of cancer formation and progression and treatment response is currently impossible to reconstitute in vitro, by computer modelling, in invertebrates or in embryonic stages. For the same reason, the complexity of the immune system is impossible to represent outside an organism.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Efficient experimental design and statistical techniques such as power analysis will keep the number of protected animals used to a minimum. Also, the ability to image tumour development in the same fish at repeated time points reduces the overall number of animals required. For example, based on numbers from previous studies, the minimum number of animals required to detect a change in the proportion of tumour bearing animals from 0.35 to 0.55 (an effect size of 0.20) with 80% power is 90 per group, testing at the 5% significance level.

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Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Zebrafish is the vertebrate of lowest neurological complexity that can be genetically modified to produce the required alterations. Unlike mouse that is commonly used for similar research, zebrafish lack the part of the brain associated with awareness and have fewer pain receptors. Further they have remarkable abilities to regenerate damaged tissues combined with potent antimicrobial activity in mucus secretions. For these reasons they arguably suffer less than mice for the same disease burden or intervention. Zebrafish will be humanely killed as soon as tumour formation or immune system modulation is sufficient to yield the desired data, which will be long before interventions can interfere significantly with feeding, locomotion, respiration or cardiovascular function, or induce significant behavioural or other physiological abnormality. Any potential suffering will be mitigated by frequent inspection and early intervention. Regarding exposure to experimental drugs, small scale pilot experiments will first be conducted to establish safety, before expanding numbers. The zebrafish are housed in a purpose-built aquarium that is maintained by dedicated and skilled staff. A vet is also available to advise on zebrafish welfare.