Date of second strand review: 26 March 2014

Project title	Cancer biology including host immunity in zebrafish		
Key words	Cancer, immunity, zebrafish		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals	Yes	
Objectives of the project	To learn more about cancer induction, maintenance and progression, including evasion of host immunity, focusing on melanoma and pancreatic neuroendocrine cancer.		
Potential benefits likely to derive from this project	1) The research will identify and validate novel drug targets for the focus cancers: cutaneous and uveal melanoma and pancreatic neuroendocrine cancer. Drug targets are the entry points for rational drug discovery and target identification is the rate limiting step in the drug discovery process. 2) The research will expand our understanding of the function of the immune system in fish, and indicate whether it is suitable for research into human disease. The research could potentially benefit the fish aquaculture industry which is trying to improve disease management in fish stocks through developing vaccines, which requires knowledge of immune system function. 3) Assuming we are successful with our efforts to advance basic understanding of the function of cell-mediated immunity in zebrafish, subsequent research could uncover what limits host immune responses to cancer. 4) The research may validate novel radioprobes that can be used in cancer diagnosis together with positron emission tomography (PET).		
Species and	45,000 zebrafish over 5 years		

approximate numbers of animals expected to be used, and anticipated period of time	
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	Zebrafish will be genetically modified and this may lead to genetic disease or cancer which could incur severe suffering (although zebrafish have much lower neurological complexity than mammals) in a limited number of animals. This will be mitigated by frequent inspection and early intervention. Zebrafish will also be vaccinated which is assumed to elicit only mild irritation.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	The involvement of multiple cell types in the process of cancer formation and progression is impossible to reconstitute in vitro or in invertebrates.
2. Reduction How the use of minimum numbers of animals will be assured	Exploratory experiments, where possible are first performed in vitro or in embryos (which are considered non-sentient). Efficient experimental design and statistical techniques such as power analysis will keep the number of protected animals used to a minimum.
3. Refinement Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	Zebrafish is the vertebrate of lowest neurological complexity that can be genetically modified to produce the required alterations. Animals will be sacrificed as soon as tumour formation is sufficient to yield the desired data streams, which will anyway be before tumours reach a size that can interfere with feeding, locomotion, respiration or cardiovascular function, or induce significant behavioural or other physiological abnormality. Likewise any genetic manipulation indicated to interfere with feeding, locomotion, respiration or cardiovascular function, or inducing significant behavioural or other physiological abnormality will result in immediate termination of the organism concerned and other animals sharing the genotype.