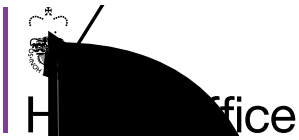

This project aims to increase our understanding of diseases and the functions of the mutated proteins that cause those diseases, as well as providing disease models for therapeutic discovery and development

The project focuses on rare inherited diseases, which together affect about one in every 10 people, even though each disease has an incidence of less than 1 in 2000 people. They frequently cause premature death in childhood and the vast majority have no approved treatments. We will also study diseases that have a more complex origin, in which the same genetic factors interact with environmental factors. There is a huge need to find treatments for both inherited and complex diseases.

We expect to have increased our understanding of the function of three or more proteins involved in childhood neurodegeneration and epilepsy, including understanding how those proteins interact with other processes within the cell and how disease arises. We plan to generate several disease models, and by studying them we will better understand the disease process. One or more chemicals will be tested to see if they treat any of the childhood neurodegenerative diseases that we are studying. We will publish our findings. We will apply for orphan drug designation for a drug to treat seizures in a childhood neurodegenerative disease called CLN2 disease. We may apply for other orphan drug designations or patents for potential drugs.

To understand the disease mechanisms, we sometimes need to manipulate other processes as well, either using pharmacological agents or by altering other genes. We then examine the fish to see if this has made a difference. We can examine their movement or behaviour, or an invisible process happening within, which we visualise with a label (often a chemical or a fluorescent tag), most often on fish that have been sacrificed for their tissue.

To search for treatments, we use embryonic disease models to screen large numbers of chemicals, and check to see if the signs of the disease are reduced by adding the chemical to the water the fish are in. If we already have good reason to believe that a chemical might make a good treatment (eg data from cellular disease models), we might use adult disease models but we will use embryonic and larval models first if possible. In this case we might bathe the fish in the chemical, or inject it. If our results are similar to those of our collaborators that have models of the same disease in other species or in cells, we can be more confident that the treatment will be beneficial for patients, or that the disease mechanism is conserved in fish.

- Killed
- Used in other projects

To model a disease, investigate the pathological steps, search for therapies and test those therapies, you need all the relevant tissues present. The diseases we work on predominantly affect the central nervous system but there is increasing evidence that they affect the peripheral nervous system, and heart, and we know that the mutated proteins function in all cells, so we expect pathology to arise in other tissues if we manage to treat the brain and improve survival. Furthermore, in some of these diseases, it is known that patients generate antibodies in the periphery that bind proteins in their brain. Hence, a full understanding or the disease and treatments will only come from studying intact animals.

We employ replacement strategies where possible. These include performing experiments in cell models (traditionally used mammalian cells, patient cells, human stem cells, the social amoeba), invertebrates such as the fruit fly and the nematode worm, frog oocytes and/or embryonic zebrafish depending on the availability of suitable models or whether the gene can be easily identified and mutated. We also take tissue from culled zebrafish and grow the cells in a dish.

These methods do not replicate the complex environment seen in a vertebrate organism or the situation at post-embryonic stages but they do give us some information so we do these experiments to inform what experiments should be performed in zebrafish at protected ages.

We expect to use up to 8000 of these fish for breeding purposes to supply embryos for experiments. This is based on 40 different strains (some are combinations of several strains, for which several intermediate strains need to be generated) with no harms caused by the genetic manipulation. Zebrafish are healthier when in groups of 20 or more, and new generations are needed about every 6 months - the old generations are not culled until the new generations are genotyped (usually as adults). For some complex strains, only a small proportion of the fish are the correct genotype but this is not

The majority of animals will be maintained as non-harmful genetically altered families, minimising

We will use the least harmful method (fluorescent microscopy or deduction of genotypes from the offspring as observed through microscopy during unregulated stages) and the earliest stage possible. When tissue samples are required, we will preferentially use skin swabs over fin clippings. Both of these methods use anaesthesia with recovery, but skin swabs are thought to be less harmful. We have recently begun using skin swabs and will need to show that the results using this method is as good as with fin clips for each strain before we can use skin swabs exclusively. We use analgesia when we take fin samples.

We aim to trial genotyping embryos < 5dpf using the Zebrafish Embryonic Genotyper, which gently removes cells from the skin for genotyping. Fish are not harmed and can be raised to adulthood without having had an invasive genotyping method.

The protocols state the minimum required monitoring provided. Monitoring will be increased if any fish respond differently than normal to the procedure.

We will follow Home Office guidance. For published methods, to ensure best practice and the most refined method is being followed before implementation, we will discuss new methods with the Named Animal Care and Welfare Officer and possibly the Named Veterinary Surgeon.

We seek advice from large zebrafish facilities and keep abreast of latest refinements via the British Association of Animal Husbandry, the European Zebrafish Meeting, the Lab Animal Science Association, the NC3Rs, the Institute for Animal Technology and the journal Zebrafish. We receive newsletters and bulletins from these groups. We search the literature for more refined methods. I am a committee member for EUFishBiomed, members of which have recently reviewed use of anaesthetic.

We have meetings every few months with other research groups using fish and the staff that care for the fish and present our research so they can feed back on any best practice they are aware of.

We will keep up to date with changes in the ARRIVE guidelines for the reporting of animal experimentation.

When we find a new method we believe to be best practice, we contact the developer and arrange for training.

Data from zebrafish can be extrapolated to other vertebrates due to a high level of conservation, particularly biochemical, cellular and developmental. Responses to pharmacological agents is also similar. This means that it is valid to use the zebrafish to model disease, investigate disease mechanisms and search for and test novel treatments. The majority of the disease models we use are for inherited neurodegenerative diseases and epilepsy, so in this case we also know how conserved

the gene is from zebrafish to humans. The rapid, external development of zebrafish enables the vast majority of experiments to be performed at developmental stages which are not regulated by the Home Office without harming the mother. Thus we feel that the zebrafish is the most refined whole animal model for our research and that, of the available vertebrate models, it has the lowest brain function.