Publishable summary

Project Title: Training Network on Zebrafish Infection Models for Pharmaceutical Screens
Project Acronym: FishForPharma

Project web page: www.fishforpharma.com
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Summary of the project objectives

Infectious diseases caused by pathogenic micro-organisms are major causes of death, disability, and social and economic disruption for millions of people. During evolution with their host these pathogens have developed intricate strategies to manipulate host defence mechanisms and outwit the immune system. To reduce the burden of infectious diseases it is important to increase understanding of these host-pathogen interaction mechanisms and to develop more effective strategies for drug discovery. The fact that zebrafish produce large amounts of embryos, which develop externally and are optically transparent, gives unprecedented possibilities for live imaging of disease processes and is the basis of novel high-throughput drug screening approaches. FishForPharma has brought together leading European research groups that have pioneered the use of zebrafish infection models and partners from the Biotech and Pharma sectors that aim to commercialise zebrafish tools for biomedical applications. The main objectives of FishForPharma were: i) To exploit zebrafish as a high-throughput model for human infectious disease research and drug development programmes; and ii) To train a new generation of young researchers with multi-disciplinary skills to introduce zebrafish models into biomedical science and pre-clinical drug screening.

Training of researchers

The Marie Curie fellows participating in the FishForPharma network included 11 PhD students and 3 early stage postdocs. They all have conducted original research projects in the area of infectious disease research using the zebrafish as a model system. Furthermore, network-wide workshops and transfers between research teams in the network have given them excellent opportunities to develop new skills and enhance their career prospects.
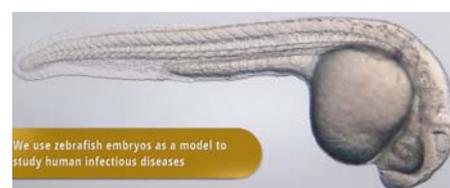
Work performed and main results achieved

Many human infectious diseases, for example tuberculosis and hospital-acquired infections can be modelled in the zebrafish. In the FishForPharma project, we further optimized and standardized zebrafish models to study bacterial and viral infections in different tissues, and a new zebrafish infection model for protozoan blood parasites was established. Transparent zebrafish embryos are ideal for live imaging of infectious disease processes. We generated new transgenic fish lines and microbial vectors with fluorescent markers to facilitate live imaging of the interactions of pathogens with host immune cells, to visualize their residence in subcellular compartments, and to provide readouts for activation of immune defences. We also produced recombinant proteins and tested antibodies for analysis of the zebrafish immune system. Zebrafish produce large amounts of embryos that develop externally and are very suitable for high-throughput screening. In this project, a high-throughput pipeline for anti-mycobacterial drug screening was optimized, integrating a commercial zebrafish breeding system, with robotic infection of embryos, and fluorescence-based automatic quantification of infection levels. RNA deep sequencing data were collected to better characterize immune responses to infections, at whole embryo and cellular level. Several of the Marie Curie fellows' research projects have concentrated on functional studies of genes involved in central pathways of the immune system. Fluorescence imaging was used to study host defence mechanisms in vivo, and virulence mutants of bacterial pathogens were investigated to gain insight into mechanisms that could be targeted for drug development. Panels of isolates from patients with different clinical outcome have been analysed for virulence in zebrafish, and results will further help to address relevant clinical questions using the zebrafish model. Clinically applied antibiotics for treatment of tuberculosis were shown to effectively inhibit bacterial growth in the zebrafish high-throughput drug screening pipeline. Promising results were also obtained with immunomodulatory drugs targeting host pathways manipulated by pathogens. The fellows at the Biotech and Pharma partners performed a chemical library screen and evaluated a set of anti-tubercular lead compounds. Several of these drugs were effective in both the mouse and the zebrafish model, but this study also revealed differences in uptake of compounds by zebrafish embryos that should be considered in future drug screens. Results achieved by the FishForPharma network have been presented at international scientific meetings and resulting publications are highlighted on the website www.fishforpharma.com, which also informs the general public about zebrafish as a model for infectious disease research.

Final results and their potential impact and use

FishForPharma could build on extensive experience of the participants with zebrafish infection models and unique robotic technology for high-throughput screening. The project resulted in: i) improved and standardized procedures for bacterial, viral and parasitic infection models; ii) an expanded toolbox for visualizing host-pathogen interactions and for analysing the host immune response; iii) a better understanding of pathogenicity mechanisms relevant to human infectious disease; iv) advanced high-throughput pipelines for drug screens; and v) proof-of-principle for antimicrobial drug discovery using zebrafish high-throughput models. The collaborative network structure of FishForPharma has facilitated the exchange of novel imaging and analysis tools and the training of young scientists in their optimal use. Insight in host immune responses has been increased by an integrated functional genomics approach combining technologies such as gene knockdown, transgenesis, and next-generation deep sequencing. Improved understanding of host-pathogen interaction mechanisms led to the identification of potential new drug targets for infectious disease treatment. Through the academia-industry collaboration FishForPharma obtained evidence that supports the use of a zebrafish tuberculosis infection model as a translational tool for anti-tubercular drug discovery. Furthermore, the project showed that the high-throughput screening pipeline for tuberculosis can be extended to models of Staphylococcal diseases, which can develop as severe complications of hospital-acquired infections. Thereby, this project has contributed to developing more efficient methods for antimicrobial drug screening, which are urgently needed due to increasing antibiotic resistances. In the longer term, the introduction of a prescreening phase in zebrafish embryos into the drug discovery pipeline may reduce subsequent screening in rodent models and accelerate the pace of drug discovery. In addition, the training of Marie Curie fellows in FishForPharma has helped to fill the growing demand for experienced researchers in the Life Sciences.