EARLY DRUG SAFETY AND EFFICACY TESTING USING THE ZEBRAFISH MODEL

Toxicity and lack of efficacy remain prominent causes of drug failure and are commonly identified during late-stage animal testing or even during early clinical testing in human subjects. As a result, there is an increasing drive in the pharmaceutical industry to reduce the expensive attrition by integrating predictive assays for both safety and efficacy in the early phases of drug discovery. In TNO’s multidisciplinary organization, the zebrafish is an excellent model to obtain early insights in the molecular mechanism of a compound in a cost effective way.

Although the zebrafish (Danio rerio) has been a well-accepted model organism in (basic) genetic and embryology research for more than forty years, the model has only been used recently for toxicity testing. The many advantages of this model compared to toxicity screening tests with larger animals have made the zebrafish an increasingly popular and acceptable organism for early drug discovery as well as efficacy screening.

These advantages compared, for instance, to using rodents are:
- Easy to breed (externally fertilized);
- Transparency (enabling visualization);
- A high number of embryos can be obtained;
- Easy to translate to human condition due to similarity of biological pathways and molecular mechanisms between humans and zebrafish;
- Experiments performed up to day five of post-fertilisation are not classified as an animal test according to European legislation.

The zebrafish is an essential test model, which is embedded in the multidisciplinary organisation of TNO (and our subsidiary TNO Triskelion). TNO can bring in several technologies and underlying scientific expertise (including systems biology, cheminformatics, biomarker identification and PK) to customise solutions for both toxicity and efficacy testing.

TOXICITY
Most zebrafish toxicity screenings are based on assessing the effects of test compounds on the morphology of the embryonic and larval development stages. TNO offers a more advanced method (see below) to provide additional in-depth information on the underlying mechanisms, which will help to validate the results obtained and design proper...
follow-up studies. This advanced model has proven to be of value in characterising and validating human disease models that are specifically designed to monitor organ specific toxicity.

EFFICACY
TNO develops models for early efficacy screening that are more cost-effective and faster than current animal studies. In addition, zebrafish studies reveal insights in the mechanism of the drug and facilitate the decision-making on which compounds to proceed with in a drug development process. We currently focus on fibrosis and metabolic syndrome (including obesity), translating our experience with preclinical mice models and related scientific knowledge of these therapeutic areas, into a zebrafish test assay.

TNO ZEBRAFISH STUDIES
For both toxicity as well as efficacy screening, TNO can offer the following elements to build your customised zebrafish study.

Morphological assessments
We routinely perform morphological assessment of embryonic and larval development at 24, 48, 72, 96 and 120 hours post-fertilisation, deriving for each time point effect concentrations (e.g. EC50s) based on a broad range of parameters that are indicative of developmental toxicity. This enables test compounds to be ranked by their potency to induce abnormalities.

Behavioural analysis
Using a ViewPoint video-tracking system we monitor and analyse the swimming pattern of larval zebrafish at 96 and 120 hours post-fertilisation as a measure of neurodevelopmental toxicity. TNO has developed a unique extension to the software for tracking the motor activity of larvae younger than 5 days post-fertilisation. This allows the motor activity test to be adjusted more accurately for the evaluation of (toxic) effects on the developing brain and thus increases the applicability domain of the zebrafish model for neurodevelopmental testing.

Histopathological analysis
We perform histopathological analysis of the zebrafish from day 1 till > 9 months post-fertilisation as well as a unique high-throughput histopathological analysis of larvae from day 3 till day 6 post-fertilisation with up to 50 larvae per slide, all equally oriented. This approach makes it possible to cost-effectively screen for, and optimally compare, the histopathological effects in each organ of all zebrafish embryos/larvae in a single experiment. TNO can assess these effects in all organs, but we are highly experienced in liver, kidney and heart.

Gross anatomy assessment
Necropsies on adult zebrafish and the isolation of any organ system of interest can be performed. Isolated organs can be used for histopathology and/or test compound analysis and -omics technologies.

Test compound analysis
We assess the concentration of test compounds and their metabolites in peripheral blood (dried blood spot analysis) and individual organs of adult fish and/or whole body preparations of embryos and larvae. This is essential in determining the actual exposure of the embryo, i.e. to exclude any false negative results of your assay by non-absorption.

Molecular profiling
TNO provides diverse -omics technologies to molecularly profile drug compounds in embryonic, larval and (organs of) adult zebrafish. In combination with our systems biology expertise, data is interpreted in a biological context. These approaches can include pathway identification, disease stage signatures or drug target discovery. In combination with our bioinformatics and cheminformatics capabilities we can assist in determining the human relevance of the target and its drugability.

Partnering
We are looking for pharmaceutical partners interested in co-developing or optimising disease models in one of our key therapeutic areas (including fibrosis, CNS, general inflammation and metabolic syndrome). In addition, we would like to extend our network of partners to jointly address and better understand bioavailability in zebrafish.

RECENT TNO PUBLICATIONS ON THE ZEBRAFISH MODEL

Normal Anatomy and Histology of the adult Zebrafish
Menke L., Spitsbergen J.M., Wolterbeek A.P.M. and Woutersen R.A.
Toxicological Pathology, 39(5), 759-775, 2011

Locomotor activity assay in zebrafish larvae: Influence of age, strain and ethanol

Zebrafish as potential model for developmental neurotoxicity testing: A mini review.

A category approach to predicting the developmental(neuro)toxicity of organotin compounds: the value of the zebrafish (Danio rerio) embryotoxicity test (ZET).
Beker-Woudenberg A., Wolterbeek A., te Brake L, Snel C., Menke A., Rubingh C., de Groot D. and Kroese D. Accepted for publication in Reproductive Toxicology 2013

Tailor-made zebrafish studies for efficacy and toxicity assessment

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