InDanio Bioscience Inc.
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Green fish open an untapped ocean for new drug discovery

InDanio Bioscience Inc.'s unique Ligand Trap system provides *in vivo*, high-content drug discovery for compounds targeting nuclear receptors and epigenetic regulators while allowing evaluation of toxicity, bioavailability and tissue selectivity.

Toronto-based InDanio Bioscience Inc. uses transgenic zebrafish (*Danio rerio*) to combine the most favorable aspects of traditional *in vitro* screening in a live vertebrate, creating a platform that is highly cost-effective, selective and fast. The company's core area of expertise is the highly druggable family of transcription factors known as nuclear receptors (NRs), which have important roles in controlling metabolism, development, growth and reproduction. Consequently, NRs offer enormous opportunities for identifying novel natural or synthetic drugs with therapeutic effects for conditions such as inflammatory, cardiovascular, neurodegenerative, reproductive and metabolic diseases, as well as most cancers. However, the discovery of novel NR-directed drugs has been challenging recently because of the lack of robust screening platforms that can assess the receptors, their cofactors and potential ligands in their normal physiological environments. Cross-reactivity between NRs and the lack of suitable assays to identify selective nuclear-receptor modulators (SNuRMs) have also resulted in dwindling numbers of approved drugs.

Selective drugs

InDanio's Ligand Trap system[1] is optimized for the identification of biologically active SNuRMs. "It allows for the simultaneous detection and evaluation of active drugs and cofactors in all tissues of a live animal," says Jens Tiefenbach, CSO of InDanio.

NR screening can be carried out in a portfolio of up to 48 transgenic NR fish lines, each expressing one of the 48 human NRs. In tissues containing appropriate cofactors and active drugs, the transparent embryos produce a green fluorescent signal. The activated NR can then be isolated from these responding tissues, together with active drugs or metabolites as well as specifically recruited protein cofactors (**Fig. 1**).

Zebrafish are the ideal vertebrate model, as they are tiny, prolific, transparent and drug absorbent. Importantly, they contain all cofactors and conditions required for the transgenic human NR proteins to function normally; all drugs tested so far have shown similar pharmacological profiles in humans and fish. The small size of fish embryos means that all screening steps can also be carried out robotically, with thousands of compounds screened quickly and comprehensively.

Homegrown pipeline

InDanio is using the Ligand Trap system to build its own pipeline of therapeutic products. Currently the company is focusing on metabolic diseases, including non-alcoholic steatohepatitis; oncology; neurodegenerative diseases; and inflammation.

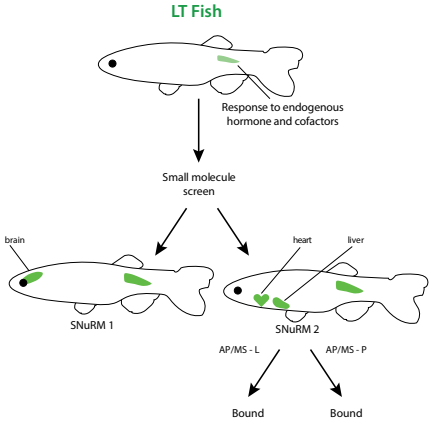

Figure 1: Overview of ligand trap fish uses. The fish denoted at the top represents a typical Ligand Trap transgenic fish showing a limited response of the human NR to host ligand(s) and cofactors. Above are two possible hits of a compound library screen showing different tissue-specific responses. The responding NR can be subjected to affinity purification and mass spectrometry (AP/MS) to identify directly bound small molecules (L) or recruited protein cofactors (P).

InDanio has three early-stage lead programs: one focusing on RAR-related orphan receptors (RORs), a second on farnesoid X receptor (FXR) and a third on peroxisome proliferator-activated receptors (PPARs). The company's lead products are (1) idebenone, a quinone originally marketed for neurological diseases that InDanio has determined to have strong potential for repurposing as a drug for fatty liver diseases (patent application number PCT/CA2014/000253); and (2) several new chemical entities with potent SNuRM activity for the highly attractive FXR and ROR families of NR targets.

Unique drug discovery capability

In addition to developing its own pipeline of products for eventual out-licensing, InDanio offers its Ligand Trap system as a source of competitive advantage for other companies in the NR- and cofactor-targeted medicine space. InDanio's Ligand Trap system is the only commercially available *in vivo*, vertebrate, high-throughput screening platform that allows screening of both activator and repressor NRs, as well as epigenetic cofactors. In the future, InDanio will have available not only a portfolio of 48 NR lines, but also a suite of cofactor lines for epigenetic drug discovery (e.g., HDAC1 and HDAC8). The Ligand Trap system's ability to also identify directly bound cofactors and ligands makes it the most comprehensive solution for NR-directed drug discovery and evaluation.

Drug discovery services include the characterization of previously identified NR-targeted compounds for toxicity, bioavailability, tissue selectivity and NR selectivity using InDanio's large portfolio of NR lines. The company is also available for *de novo* NR drug discovery using customer-supplied or in-house compound libraries, as well as for custom services such as developing assays for proteins of interest and characterizing various activities using transient RNA injection assays.

> **"We are open to establishing all types of collaborations surrounding our portfolio**
>
> Anne Cheung, CEO

Access to InDanio's drug discovery platform can be arranged through a number of different business partnership models: screening contracts, target-validation services, or broader research collaborations entailing custom assay development integrated with the existing Ligand Trap system. "We are confident our Ligand Trap system will enhance the success of NR-targeted and epigenetics drug discovery programs. We are open to establishing all types of collaborations surrounding our portfolio of NR or cofactor lines, as well as our pipeline product opportunities," says Anne Cheung, CEO of InDanio.

1. Tiefenbach, J. *et al. PLoS ONE* **5**, e9797 (2010).

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