MULTITOX ASSAY

REDUCTION OF DRUG ATTRITION AND ZEBRAFISH

The pharmaceutical industry is under growing pressure to improve R&D productivity to sustain sufficient innovation to replace the loss of revenues due to patent expiration for successful products. Combined with the low number of new molecular entities (NMEs) entering into clinical phases, new screening strategies are demanded. Compounds fail for many reasons, but some are more avoidable such as poor oral bioavailability, pharmacokinetic properties or toxicity, and low margins of safety.

A new strategy to reduce attrition and maintain the number of NMEs entering clinical phases requires improving toxicity characterization at early stages of drug discovery combined with more efficient and cost effective assays.

The zebrafish is emerging as a complement to existing *in vitro* technologies and established preclinical *in vivo* models that can be scaled for high-throughput. Technological innovation has helped the zebrafish embryo gain ground as a disease model and an assay system for drug screening. Moreover, the zebrafish embryo offers a cost effective system that combines many features that are desirable for the development of new approaches to drug development.

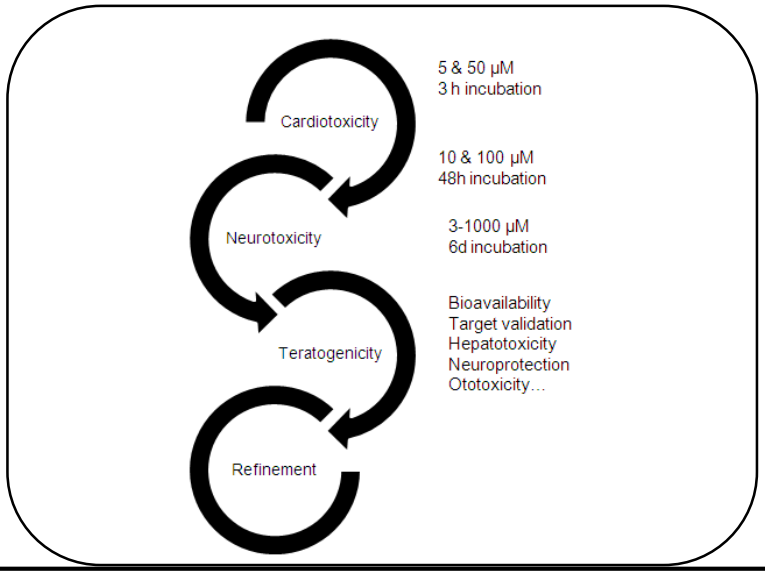
By using Zebrafish toxicity assays not only for predicting toxicities in later stages but applying them at the very early stages of the Drug Discovery process, it would be feasible to improve the selection of safe candidates or to understand potential toxicities that should be evaluated, thereby decreasing the attrition of drug candidates in more advanced phases due to toxicity.

MULTI TOXICITY ASSAY

Based on the organ attrition related to specific toxicities and its incidence in the Drug Development process, we propose an innovative approach sequentially combining different toxicity assays, to rapidly address the deselection of compounds through toxicity assessment in zebrafish embryos. This approach will also offer the reduction of costs linked to a reduction of compounds tested at each sequential assay.

Biobide has developed a multiassay that combines three mayor toxicity assays responsible for attrition in the R&D process: Cardiotoxicity, Neurotoxicity and Teratogenesis, followed by a refinement step that is open to the specific requirements of each screening program.

Scheme of the assays and conditions used for the MultiTox Assay

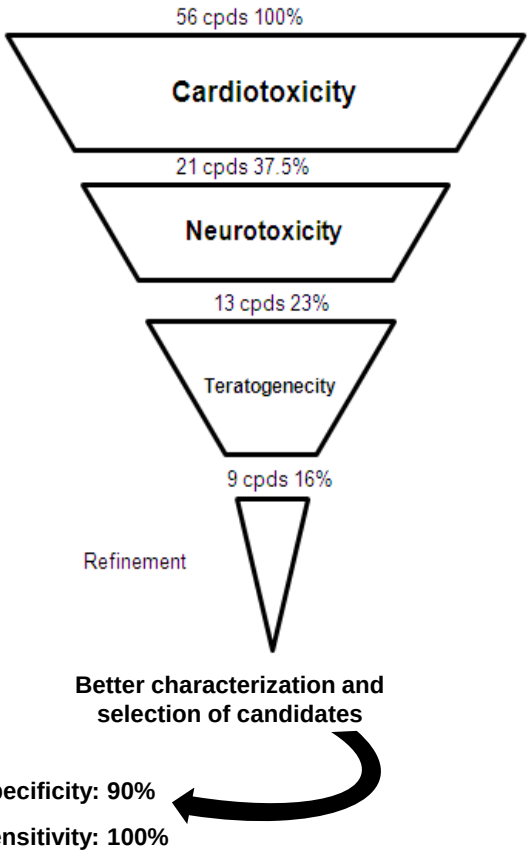


Out of 56 tested reference compounds, 9 compounds did not show any toxicity at the end of the process. In figure 1 the number of compounds selected in each step of the assay are shown. The assay has an overall sensitivity of 90% and specificity of 100%.

Figure 1. List of reference compounds used in the validation process

Classification of products	Reference compounds	Classification of productos	Reference compounds
Toxins	Aflatoxin	Beta-blockers	Propanolol
Nootropic	Deprenyl		Timolol
Analgesic	Acetaminophe		Sotalol
Statins	Lovastatin	Antipsychotics	Thioridazine
	Simvastatin		Haloperidol
	Mevastatin		Pimozide
Anti-inflammatory/immunosupressant	Dexamethasone		Sertindole
	Tetracyclin		Risperidone
Antibiotics	Penicillin G	Anti-convulsant	Carbamazepine
Anticoagulant	Warfarin		Valproic acid
Gastrointestinal agents	Cisapride	Colinergic agonist	Pilocarpine
Calcium blockers	Verapamil		Nicotine
	Diltiazem		Tacrine
	Nitrendipine	Antihistaminics	Astemizole
	Terodiline		Terfenadine
Antitumorals	Tamoxifen	Anti-infective	Halofantrine
	Chlorambucil		Foscarnet
	5-Fluorouracil		Mefloquine
	Thalidomide		Isoniazid
	Hydroxyurea	Hormones	Testosterone
Antidepressants	Fluoxetine		Estradiol
	Amitriptyline		Hydrocortisone
Antiarrhythmics	Lidocaine	Vitamins/anti-oxidants	Ascorbic acid
	Flecainide		NAC
	Propafenone	Others	Sucrose
	Amiodarone		Indirrubin-3-oxine
	Disopiramide		Digitoxin
Ina openers	sdz-201106		Ketanserine

Figure 2. Percentage and the number of compounds selected in the multiassay approach for a better selection of candidates.



- The MultiTox assay can help to decrease the attrition rate linked to toxicity using *in vivo* data at early stages of the Drug Discovery Process by revealing which compounds do not induce any of the main toxicities.
- The throughput of the assay is now set to a hundred compounds per week.
- The use of this multiassay approach would result in a 50% reduction of the time and cost needed to perform each assay individually.
- The MultiTox assay is modular and can be customized to any requirement. It allows changing the type, the sequence and even the conditions of each toxicity assay selected for the multiassay approach.

Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, Schacht AL. Nat Rev Drug Discov. 2010 Mar;9(3):203-14.
McKim JM Jr.. Comb Chem High Throughput Screen. 2010 Feb;13(2):188-206.
Eckert H, Bajorath. Drug Discov Today. 2007 Mar;12(5-6):225-33. Epub 2007 Feb 7. Review.
Chakraborty C, Hsu CH, Wen ZH, Lin CS,. Curr Drug Metab. 2009 Feb;10(2):116-24. Review.