ZeGlobalTox: An Innovative Approach to Address Organ Drug Toxicity Using Zebrafish

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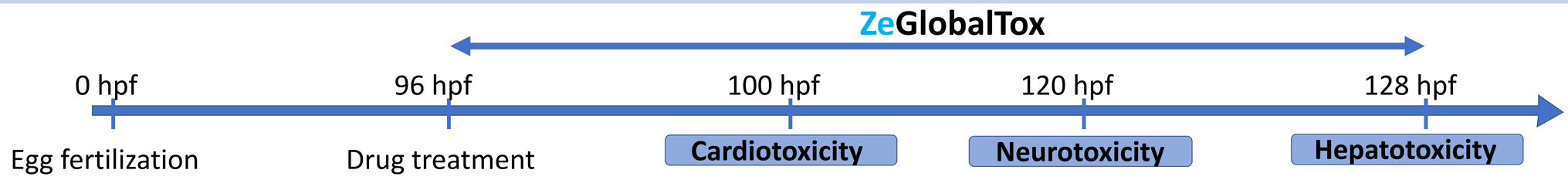
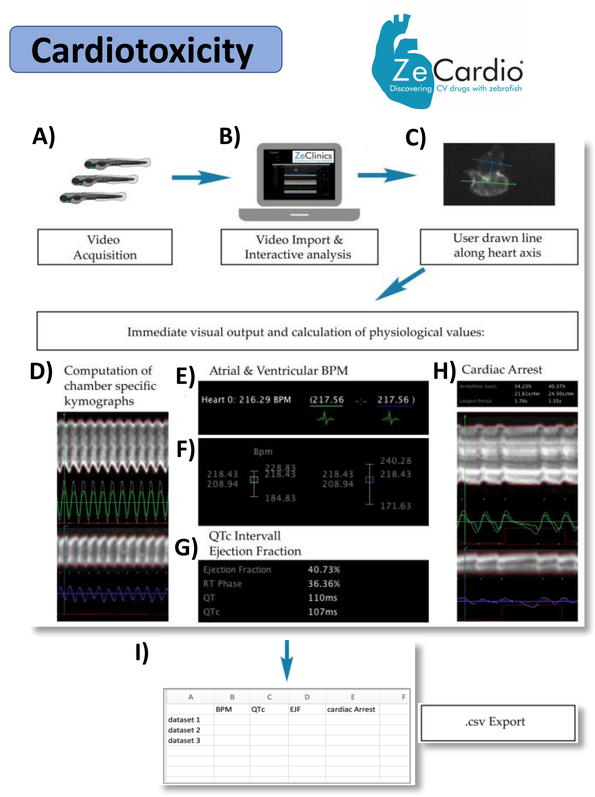
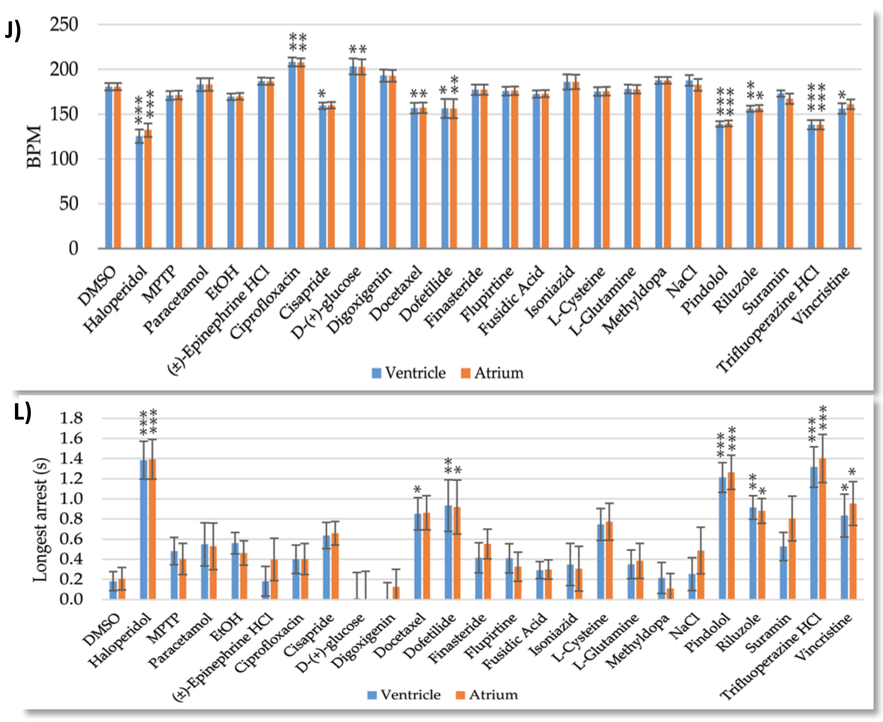
Toxicity is one of the major attrition causes during the drug development process. In that line, cardio-, neuro-, and hepatotoxicities are among the main reasons behind the retirement of drugs in clinical phases and post market withdrawal. Zebrafish exploitation in high-throughput drug screening is becoming an important tool to assess the toxicity and efficacy of novel drugs. This animal model has, from early developmental stages, fully functional organs from a physiological point of view. Thus, drug-induced organ-toxicity can be detected in larval stages, allowing a high predictive power on possible human drug-induced liabilities. Hence, zebrafish can bridge the gap between preclinical in vitro safety assays and rodent models in a fast and cost-effective manner.

ZeGlobalTox is an innovative assay that sequentially integrates in vivo cardio-, neuro-, and hepatotoxicity assessment in the same animal, thus impacting strongly in the 3Rs principles. It Reduces, by up to a third, the number of animals required to assess toxicity in those organs. It Refines the drug toxicity evaluation through novel physiological parameters. Finally, it might allow the Replacement of classical species, such as rodents and larger mammals, thanks to its high predictivity (Specificity:89%, Sensitivity: 68% and Accuracy: 78%).



Cardiotoxicity





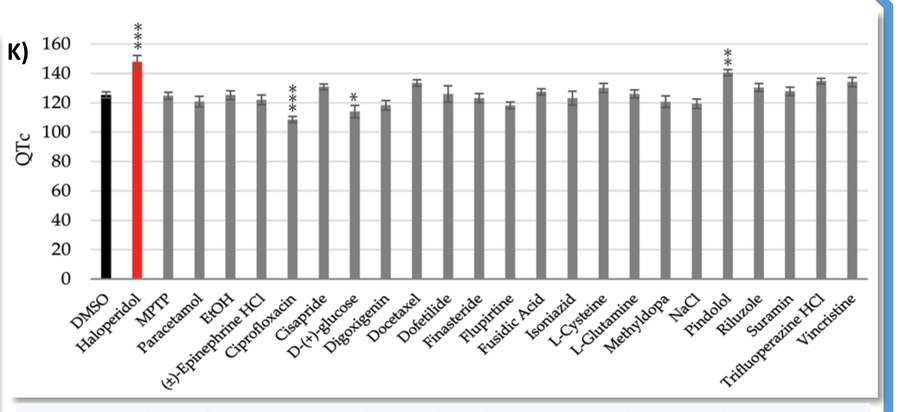


Fig 1. ZeCardio software user pipeline and cardiotoxicity results. (**A**) Video acquisition of larvae incubated with the candidate drug; (**B**) Video import into the software; (**C**) User drawn line along the heart axis; (**D–H**) GUI (Graphical User Interface) display of (**D**) Chamber kymographs; (**E**) atrial and ventricular BPM (Beats Per Minute) values; (**F**) Distribution plot over time of atrial and ventricular BPM; (**G**) QTc interval and EJF (Ejection Fraction) values and (**H**) Cardiac arrest events; (**I**) Output values are presented in .csv format. Kymographs and measurements are displayed in green or blue for ventricle or atrium respectively. (**J**) Bar graphs showing heart beat frequency in beats per minute (bpm); (**K**) QT corrected interval (QTc) (**L**) and longest cardiac arrest of 100 h old zebrafish larvae. Asterisks indicate statistical significance after a One-way ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Black bar: negative control. Red bar: positive control. n = 16 but for DMSO n = 46.

Neurotoxicity

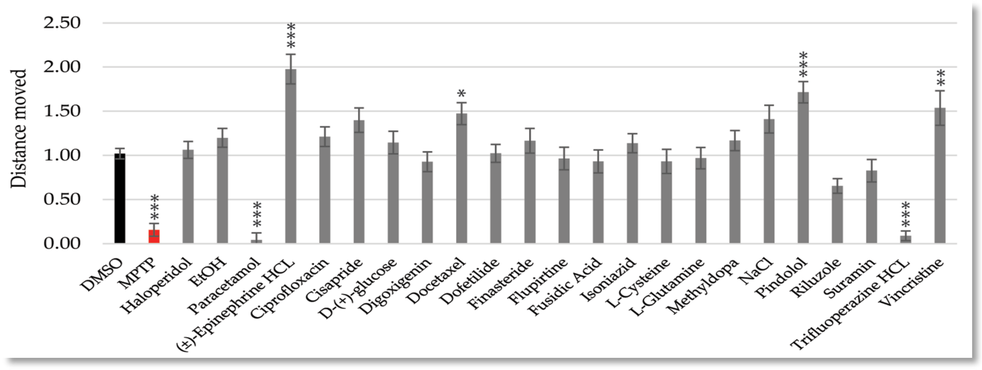
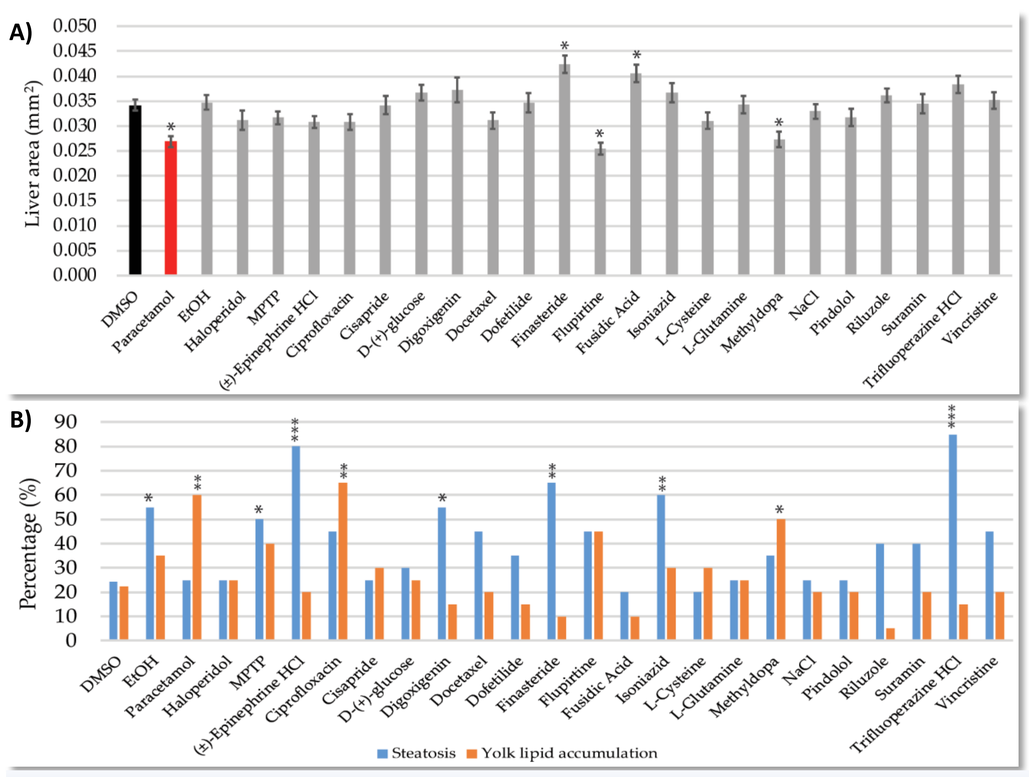


Fig 2. Locomotion results. Bar graphs showing total distance moved corrected to the DMSO group. Asterisks indicate statistical significance after a One-way ANOVA * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Black bar: negative control. Red bar: positive control. Experiment performed once with 16 larvae per condition. $n = 43$ for the DMSO

Hepatotoxicity



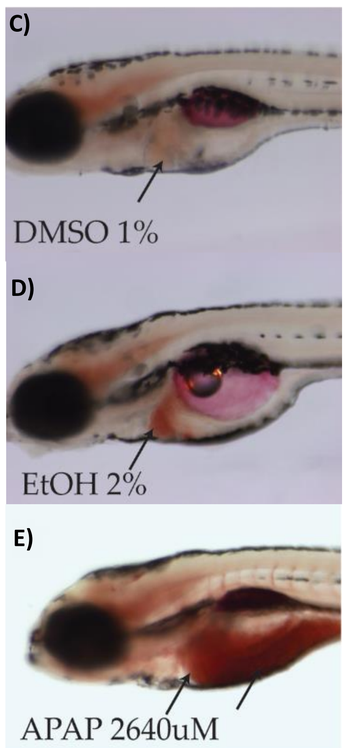


Fig 3. Hepatotoxicity results. (**A**). Bar graphs showing average liver area in mm. (**B**) Bar graphs showing the percentage of larvae presenting steatosis or yolk lipid accumulation after oil red O stain (**C–E**) Representative oil red O whole mount staining images of (**C**) DMSO, (**D**) EtOH and (**E**) APAP; black arrows point at non-affected liver (**C**), liver with steatosis (**D**), and yolk lipid retention (**E**), respectively. Asterisks indicate statistical significance after One-way ANOVA (liver area) or Fisher's exact test (steatosis and yolk lipid retention): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Black bar: negative control (**A**). Red bar: positive control (**A**). n = 20 but for DMSO n = 45.

Conclusions

ZeGlobalTox is highly predictive, demonstrating its validity as an innovative tool for integrated multi-organs toxicity prediction of new drugs during preclinical drug discovery phases and, additional, for the implementation of 3Rs recommendations.

		Cardiotoxicity				Neurotoxicity				Hepatotoxicity	
		Human				Human				Human	
		-	+			-	+			-	+
Zf	-	TN: 9	FN: 4	Zf	-	TN: 11	FN: 6	Zf	-	TN: 10	FN: 2
	+	FP: 1	TP: 9		+	FP: 0	TP: 7		+	FP: 3	TP: 8

	Cardiotoxicity	Neurotoxicity	Hepatotoxicity	ZeGlobalTox
Specificity	90%	100%	77%	**89%**
Sensitivity	69%	54%	80%	**68%**
Accuracy	78%	75%	78%	**77%**
PPV	90%	100%	73%	**88%**
NPV	69%	65%	83%	**72%**

Specificity (TN/(TN+FP), Sensitivity TP/(TP+FN), Accuracy (TP + TN)/(TP + TN + FP + FN); PPV: TP/(TP + FP); NPV: TN/(TN + FN). **Zf:** zebrafish, **PPV**: positive predictive value, **NPV**: negative predictive value, **TN:** true negative, **TP:** true positive, **FN:** false negative, **FP:** false positive.

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