Adaptation of a Human Pluripotent Stem Cell Assay for Developmental Toxicity Screening

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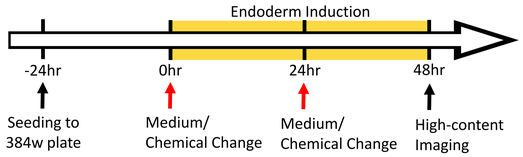
Background

- The Human Pluripotent Stem Cell Test (hPST) utilizes stem cell endoderm differentiation to identify teratogens that perturb SOX17 expression (1).
- To adopt the principles of the hPST, we utilized the RUES2-GLR (germ layer reporter) cell line engineered with fluorescent reporters for biomarkers representing all three gastrulation lineages: endoderm, ectoderm and mesoderm (2).
- This RUES2-GLR assay enables rapid 384-well high-throughput screening and quantitative analysis of multi-lineage differentiation, demonstrating potential to identify and prioritize teratogens based on affected gastrulation lineages.

Method

- Human pluripotent stem cell line (RUES2-GLR) containing a fluorescent biomarker, SOX17–tdTomato (endoderm), was used to assess perturbations during endoderm differentiation.



- High-content image analysis was used to quantitatively determine the percentage of SOX17+ cells and total cell count (viability).

References

1) Sei Kameoka, Joshua Babiarz, Kyle Kolaja, Eric Chiao, A High-Throughput Screen for Teratogens Using Human Pluripotent Stem Cells, Toxicological Sciences, Volume 137, Issue 1, January 2014, Pages 76–90.
2) Martyn, I., Kanno, T.Y., Ruzo, A. et al. Self-organization of a human organizer by combined Wnt and Nodal signalling. Nature 558, 132–135 (2018).

This poster does not necessarily reflect EPA policy.

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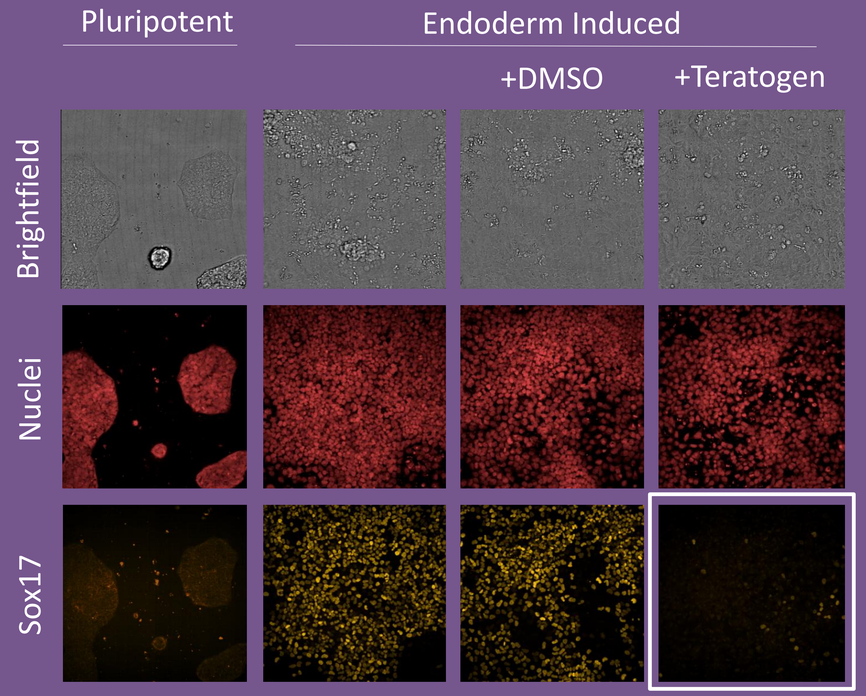
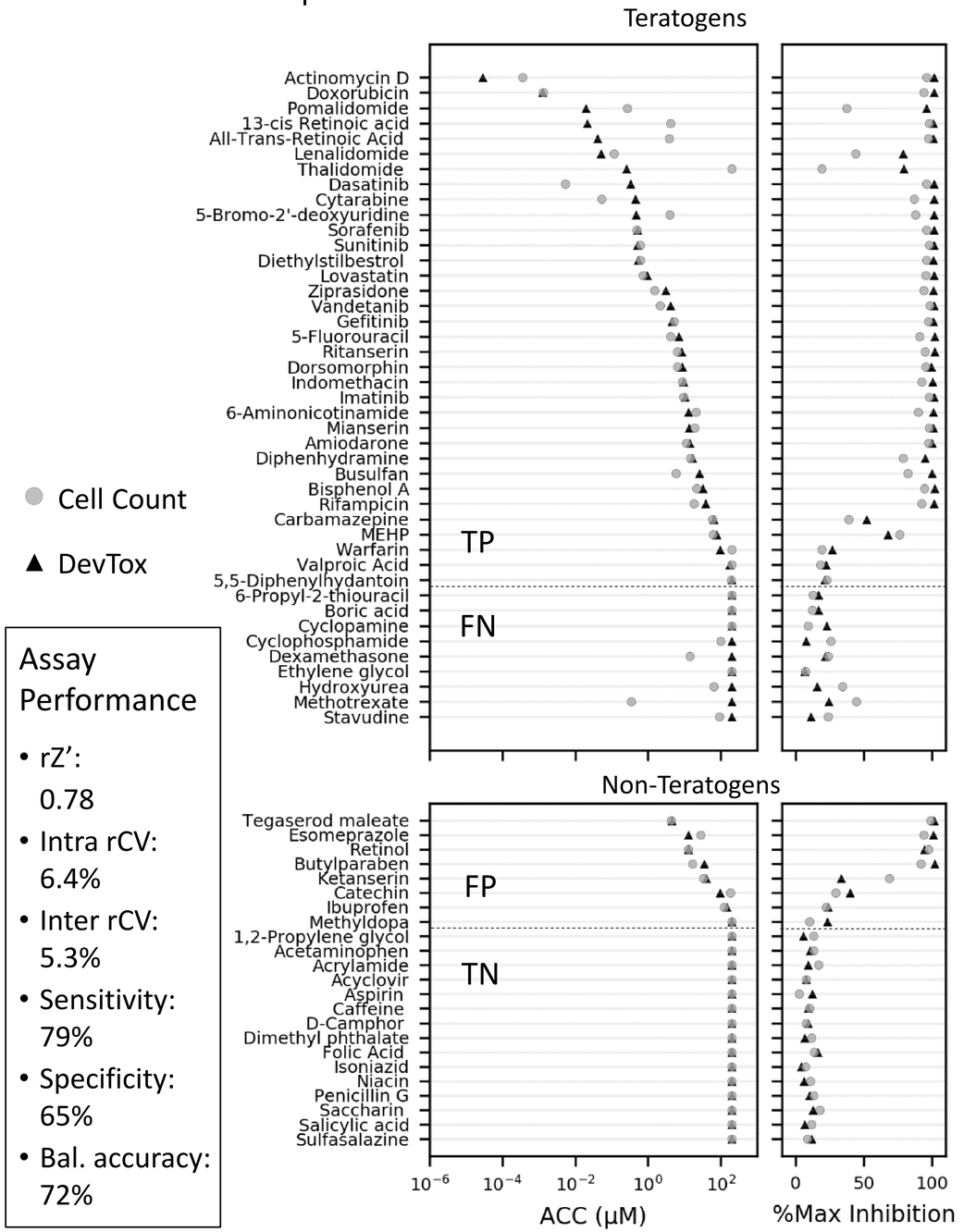


Inhibition of SOX17 expression during endoderm induction is more predictive of a developmental toxicant than cytotoxicity.

Evaluation of assay predictivity using a 66 chemical training set with known developmental effects.



Assay Performance

- rZ': 0.78
- Intra rCV: 6.4%
- Inter rCV: 5.3%
- Sensitivity: 79%
- Specificity: 65%
- Bal. accuracy: 72%