### Introduction

Assessment of the developmental toxicity potential of new chemicals is both resource-intensive and time-consuming. Large numbers of laboratory animals are required and the predictive value of these decades-old tests has been challenged. Availability of more predictive developmental toxicity screens would reduce costs and increase pharmaceutical and chemical safety. A small molecule biomarker-based in vitro assay was developed using human induced pluripotent stem cells (iPS cells) and two metabolites (cystine and cysteine), previously identified as biomarkers of teratogenicity in human embryonic stem (hES) cells. The assay uses the ratio of the two metabolites (o/c ratio) to indicate the concentration at which a test compound may perturb cellular metabolism in a manner indicative of teratogenicity.

Our goal was to determine if the assay could be migrated to an iPS cell-based model by testing whether the cells respond to chemical insult in the same manner as hES cells. iPS cells are derived from the genetic manipulation of human somatic cells and are being widely investigated for use in place of hES cells as a less controversial model. While human iPS cells are phenotypically and genetically similar to hES cells in many respects (i.e., morphology, proliferation, gene expression), recent research has revealed that numerous subtle but important molecular differences exist. We tested 31 known compounds (23 training and 8 test set compounds) in both hES and iPS cells. The predictions (teratogen vs. non-teratogen) as well as the concentration at which a compound was predicted teratogenic were compared between the two cell lines. The transition of the targeted biomarker assay to iPS cells harnesses the predictive power of the hES cells without the ethical controversy surrounding them.

### Methods

**Cell Culture**

- Established hESC and iPS cell lines for electrophysiology and neurobiology.
- Established iPS cell lines using nuclear transplantation and episomal vectors.
- Expanded hESC and iPS cell lines to ~3 x 10⁶ cells in 96-well plates.
- Established hiPSCs and hiESCs using episomal vectors.

**Sample Preparation**

- Prepared samples for electrophysiology and neurobiology.
- Prepared samples for microscopic analysis.
- Prepared samples for biochemical analysis.

**Data Analysis**

- Analyzed data using statistical software.
- Analyzed data using machine learning algorithms.
- Analyzed data using gene expression analysis.

**Results**

- A set of short experiments were performed to determine if the hES assay parameters developed for hES cells would apply to iPS cells.

### Question 1: Do iPS cells attach with the same efficiency as hES cells?

- CellsWell vs. Hours after Plating
- CellsWell vs. Hours after Plating

### Question 2: Do iPS cells double at the same rate as hES cells in our 96-well culture?

- Cell number was equivalent between iPS and hES cells 24 hours after plating.
- Additionally, both cell lines underwent ~3 doublings during the 96 hour culture period.

### Question 3: What is the optimum treatment length for iPS cells?

- The interpolation concentration where the o/c ratio crosses the teratogenicity threshold (i.e., Teratogenic Potential) for all test compounds was selected as the treatment period in the iPS cell-based developmental toxicity assay.

### Conclusions and Future Directions

- The current study shows proof of concept that the assay can be transferred from an hES cell-based model to iPS cells.
- 27 of the 31 compounds have the same prediction in iPS and hES cell assays.
- Using a teratogenicity threshold of 0.88 for the o/c ratio (determined with hES cell data), the iPS cell-based assay had an accuracy of 81% for classifying potential developmental toxicants.
- Ongoing research will further define the teratogenicity threshold in the iPS cell-based assay to account for subtle differences in response between the two cell lines.

We recently published the hES cell data used for comparison in both hESC and iPSC.

### Acknowledgements

We gratefully acknowledge the National Science Foundation (NSF SBIR Phase II and IIB Award #1065835) for funding this study and our collaborators at Agilent Technologies for providing technical assistance, software and instrumentation.