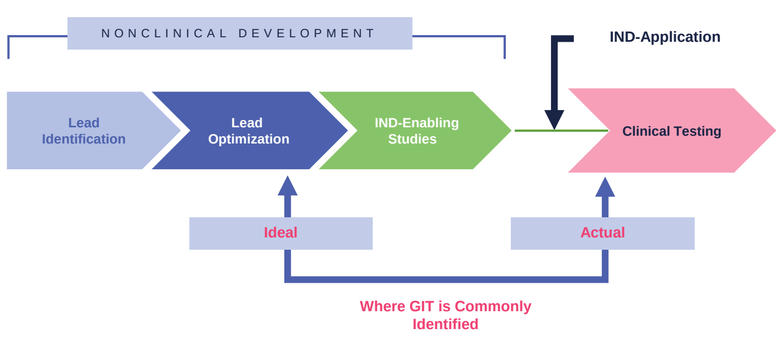
A Sensitive and Specific Human Primary Stem Cell-Based Assay for Predicting Diarrheagenic Potential of Therapeutic Agents

James Levi, Lauren Boone, Swetha Peddibhotla, Colleen Pike, Reganne Lorichon, Mariana Castillo, William Thelin, Maureen Bunger, Liz Boazak

Altis Biosystems, Durham, NC, 27709

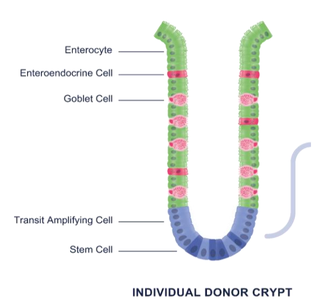
Need for Detecting Intestinal Drug Toxicity

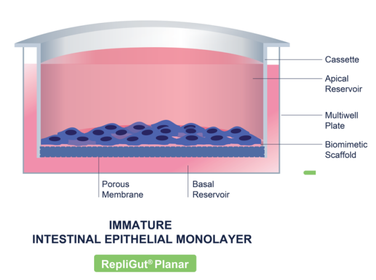


Diarrheagenic potential of new immuno- or chemotherapies is poorly predicted using animal models. Our goal is to build a human-relevant in vitro model to complement and improve current approaches to GI adverse event prediction for enhanced clinical safety profiling.

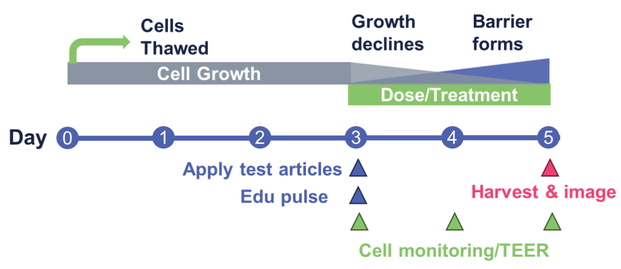
RepliGut® StemTox™ Overview

A multi-endpoint assay for intestinal toxicity





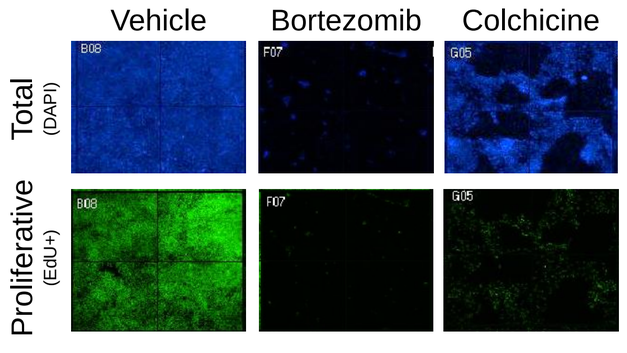
RepliGut® Planar is a unique, human stem cell derived platform that recreates the colonic epithelium and enables biologically relevant screening of compounds and disease modeling.

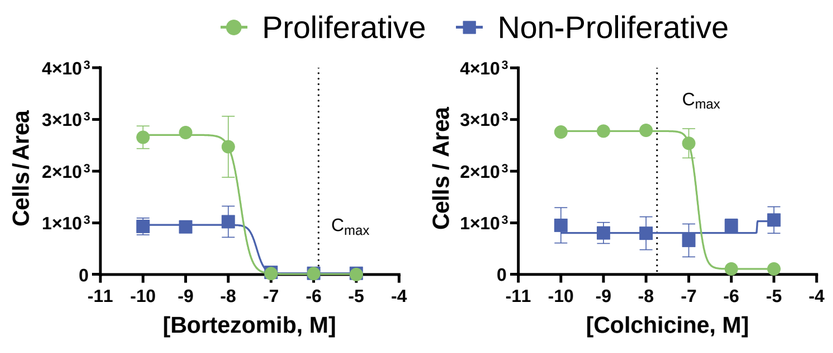


This five-day assay predicts GI toxicity by assessing two major functions of the intestinal epithelium: 1) barrier formation (via TEER measurements), and 2) cell health (via high-content imaging). Dose response curves are generated for each endpoint yielding quantitative readouts that can be compared to clinical plasma C_{max}.

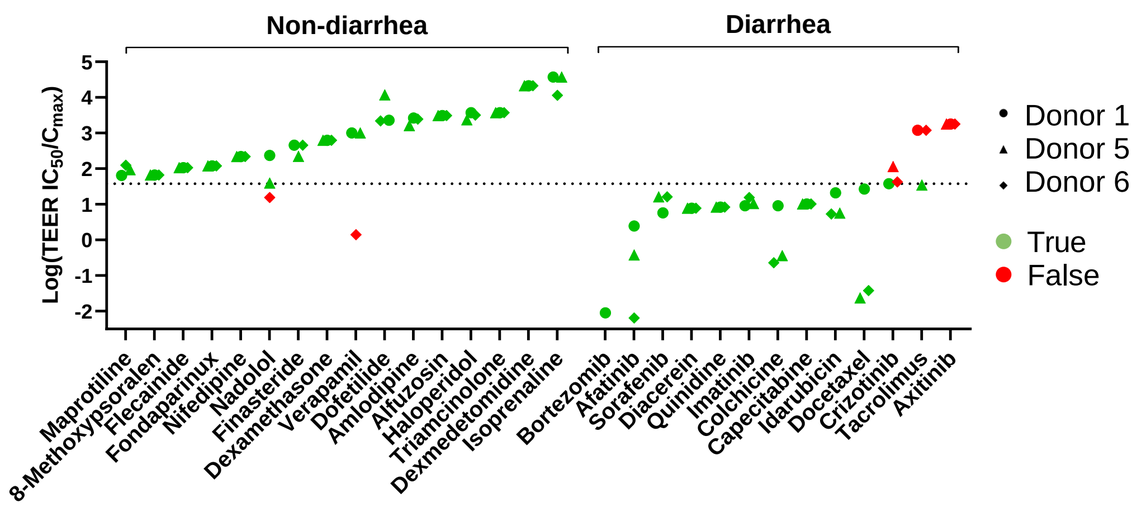
Sensitive Detection of Cellular Toxicity

RepliGut® StemTox™ Assay is sensitive to cell proliferation selective and non-selective drugs. Cells plated in 96-transwell plates were treated with bortezomib (nonselective) or colchicine (selective). Total cell count (DAPI, blue) and proliferative cell count (EdU+, green) were measured 48 hours post exposure. Bortezomib treatment decreased both cell populations. Colchicine showed toxicity towards proliferative cell populations (green line) while total non-proliferative cells were unaffected (blue line).





Accurate Prediction of Diarrheagenic Potential



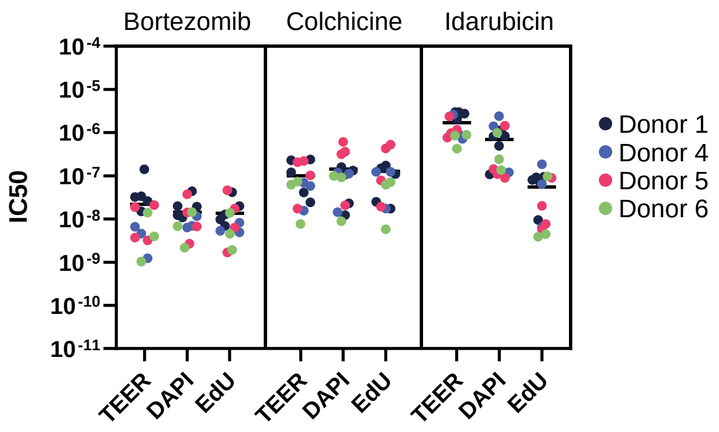
StemTox™ Assay differentiates between marketed diarrheagenic- and non-toxic drugs. Cells from three donors were plated in 96-transwell plates and TEER was measured 48 hours post drug exposure. Each drug is plotted against its IC50 to clinical Cmax ratio. Classification threshold (dotted line) was determined by ROC analysis. Red points denote false positives or false negatives for non-diarrheagenic and diarrheagenic categorized drugs, respectively.

Model	Readout	Cmpds	Sensitivity	Specificity	Accuracy
StemTox™ Donor 1	TEER	29	85%	100%	93%
StemTox™ Donor 5	TEER	29	85%	100%	93%
StemTox™ Donor 6	TEER	29	77%	88%	83%
Microtissue[1]	TEER	29	85%	94%	90%
Microtissue[1]	TEER	29	77%	94%	86%
Microtissue[1]	MTT	29	54%	100%	79%
Caco-2[1]	TEER	29	62%	100%	83%
Ileal Organoid[2]	CTG	29	91%	100%	96%

StemTox™ Assay exhibits similar or better toxicity detection over intestinal cell culture models. Measures of diagnostic accuracy for 29-drug reference set calculated for three independent executions of StemTox™, compared to diagnostic accuracy for several published executions (Ileal organoids, MatTek GI microtissues, and Caco-2 cells)[1,2].

[1] Peters, M. F., et al. (2019). Human 3D Gastrointestinal Microtissue Barrier Function As a Predictor of Drug-Induced Diarrhea. *Toxicological sciences*, 168(1), 3–17. [2] Belair, D. G., et al. (2020). Human ileal organoid model recapitulates clinical incidence of diarrhea associated with small molecule drugs. *Toxicology in vitro*, *68*, 104928.

Donor Diversity and Reproducibility



Donor Characteristics				
Donor	Age (yr)	Sex	Race	Weight (lbs)
1	23	Male	Caucasian	182.1
4	51	Female	Caucasian	158.4
5	50	Male	African American	162.0
6	51	Male	African American	219.0

Consistency of StemTox™ Assay across four human donors. (Left) Cells from four donors were plated in 96-transwell plates and TEER, cell count (DAPI) and EdU incorporation were measured 48 hours post exposure. Each data point represents an independent experiment (n=15).

Conclusions

These findings confirm ability of RepliGut® Planar culture systems to:

- Discern toxicities to proliferating vs fully differentiated compartments of the intestinal epithelium
- Correctly identify drugs associated with clinical diarrhea incidence versus negative controls
- Perform with similar or better accuracy than competing commercially available models

RepliGut® StemTox™ Assay provides an efficient and informative in vitro screen to predict diarrheagenic potential that integrates sensitive and robust endpoints, 96-well plate testing capabilities, and insights into underlying mechanisms of toxicity.

Visit altisbiosystems.com