A sensitive and specific human primary stem cell-based *in vitro* assay for predicting gastrointestinal toxicity risk of therapeutic agents

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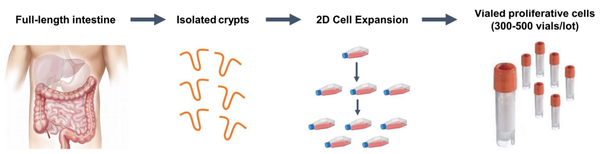
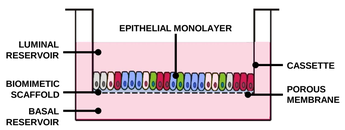
Predicting the GI toxicity risk of therapeutic agents

- Human intestinal stem cell (ISC)-derived culture systems offer several advantages for studying gastrointestinal diseases
  - Replicates human physiology
  - Reduces the need for animal-based preclinical studies
  - Allows for high throughput assay design
  - Reduces downstream timelines and costs
- The RepliGut® Planar platform can be utilized to assay known therapeutics that cause adverse events in the clinic (i.e., diarrhea) and can produce robust dose response curves used to predict these events
- This assay integrates a 6-log dose range to produce a dose-responses for the drug-induced toxicity of Idarubicin, Bortezomib, Colchicine, Afatinib, and Docetaxel (positive controls) versus Nadolol and Verapamil (negative controls) within a 10-fold range of the C_{max}

RepliGut® Planar

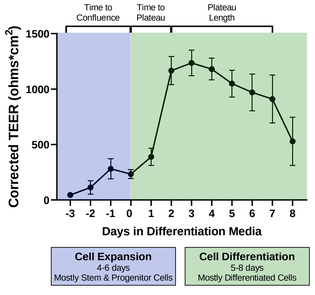
RepliGut® Planar is a unique, stem cell derived platform that recreates the human colonic epithelium and enables biologically relevant screening of compounds and disease modeling

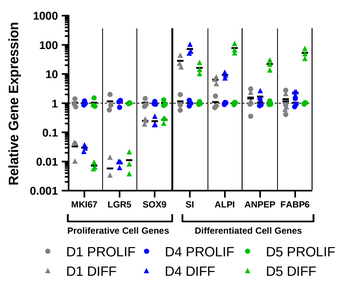




The transwell format allows for easy access to apical and basolateral compartments for individual compound addition or supernatant analysis

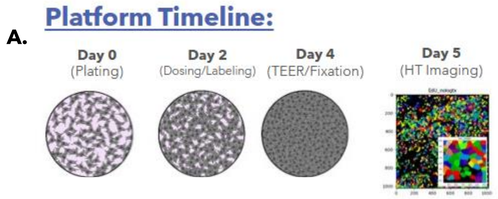
10-day culture timeline allows for investigation and analysis of proliferative or differentiated cell populations

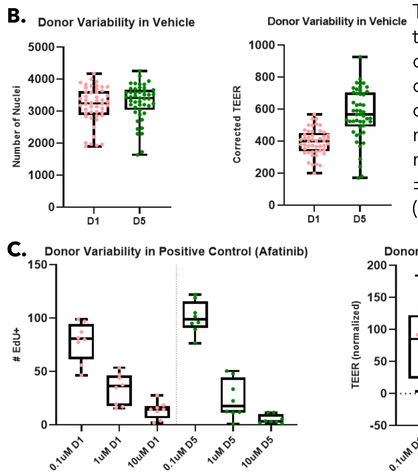




The RepliGut ® Planar cell culture timeline contains a cell expansion phase (4-6 days) then a cell differentiation phase (5-8 days) that can be monitored via TEER. Gene expression of 3 human donors shows cells in the differentiation phase have downregulated proliferative cell genes and upregulated for differentiated enterocyte cell genes relative to cells in the proliferative phase. D = Donor ; PROLIF = proliferative cells ; DIFF = differentiated cells

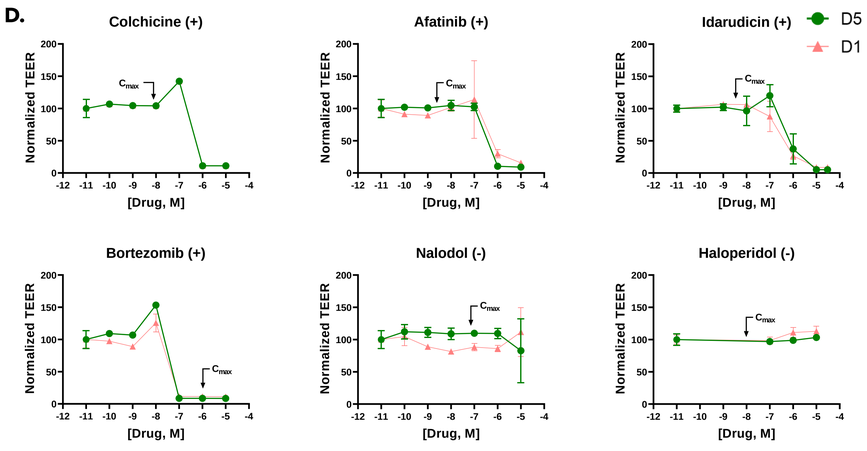
GI tox assay utilizing RepliGut® Planar





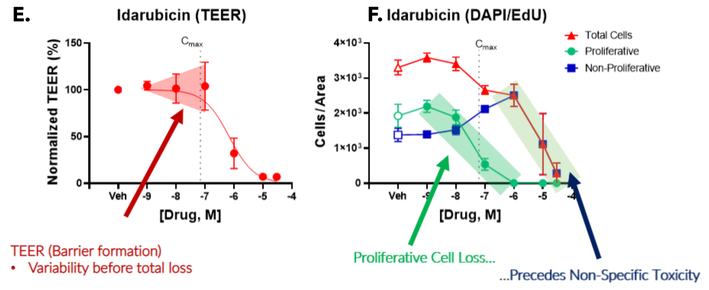
A. The GI toxicity assay duration is four days with therapeutic dosing on Day 2 and the TEER/fixation on Day 4. On Day 5, high-throughput imaging will be conducted to determine non-proliferative and total cell counts. **B.** Donor variability in the vehicle control is represented by corrected TEER and number of nuclei; n=8 technical replicates; n=6 biological replicates; Error bars represent ±SD. **C.** Donor varability in the Positive control (Afatinib) is represented by average corrected TEER and % positive Edu cells; n=8 technical replicates; Biological replicates n=2 for each treatment; Error bar are represented by ±SD.

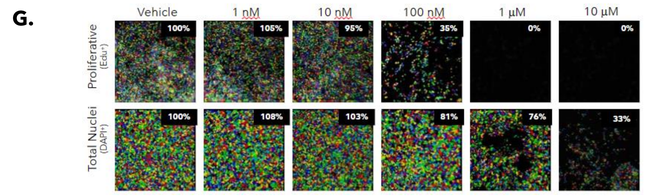
Assessment of barrier function as an index of dose-effects



Comparison of dose responses across two human donors. Passage 10 transverse colon epithelial cells from multiple donors were plated in 96-transwell plates and TEER was measured every 48 hours post exposure from a given therapeutic. Dose curves were constructed, and no significant differences were observed between each donor's response to the therapeutic(s). Four positive incident drugs, Colchicine, Afatinib, Idarubicin, and Bortezomib, and two negative incident drugs, Nadolol and Haloperidol, were tested.

Utilizing proliferative and total nuclei counts as an index of dose-effect





Investigating the dose response when ISCs are exposed to Idarubicin. The variability before total loss of TEER can be shown by figure **E**, and this variability is represented by the selective toxicity towards the proliferative population shown in figure **F**. Figure **G** represents the cell count enumeration to determine the total cell count.

Comparing TC_{15} calculations across donor responses and measures

H.

	Compound (Mechanism)	Diarrhea Incidence	Clinical C_{max}	Altis TEER TC_{15} D1	Altis Prolif. TC_{15} D1	Altis Non-Prolif. TC_{15} D1	Altis TEER TC_{15} D5	Altis Prolif. TC_{15} D5	Altis Non-Prolif. TC_{15} D5
Positive	Bortezomib (Proteosome Inh)	77%	1.3E-06	8.63E-09	3.53E-09	6.53E-09	9.95E-09	5.77E-09	6.76E-09
Positive	Colchicine (Microtubule Inh)	96%	1.8E-08	1.08E-07	5.38E-08	1.45E-08	1.08E-07	6.92E-08	4.84E+04
Positive	Afatinib (EGFR Inh)	72%	7.8E-08	1.19E-07	7.98E-08	1.04E-07	7.06E-08	6.70E-08	7.76E-08
Positive	Idarubicin (DNA Intercalation)	51%	8.8E-08	6.07E-08	8.01E-10	3.35E-11	1.45E-07	5.33E-10	1.64E-07
Positive	Docetaxel (Microtubule Inh)	42%	3.7E-06	1.25E-07	1.16E-09	1.1E-07	6.51E-08	1.82E-09	4.76E-09
Negative	Nadolol (Beta Blocker)	0%	430 nM	>1.76E-06	>1.76E-06	>1.76E-06	1.76E-06	1.76E-06	1.76E-06
Negative	Verapamil (Ca2+ Channel Inh)	2%	99 nM	>1.76E-06	>1.76E-06	>1.76E-06	1.76E-06	1.76E-06	1.76E-06

Comparing TC_{15} between donor and other commercially available platforms. Altis calculated the TC_{15} from the TC_{50} using the equation shown in figure **I**. All values generated by Altis were noted as true positive or true negative due to being 20-fold from the TC_{15} /C_{max} and are shown in figure **H**. the TC_{15} was calculated by: $\mathrm{TC_F} = \frac{\mathrm{F}}{(100-\mathrm{F})}^{1/\mathrm{H}} * \mathrm{TC_{50}}$, where H = Hill Slope, and F = 5 inhibition of interest.

Conclusions and future directions

- The RepliGut ® Planar cell culture platform creates competent dose responses for known therapeutic compounds that are superior to current offerings in the space
- Moving forward other regions and donors will be investigated for their competency for assaying known therapeutics utilizing RepliGut ® Planar

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