RepliGut® 2D Crypt Platform Enables Long-term Drug Treatment and Washout Studies on Proliferative and Differentiated Human Transverse Colon

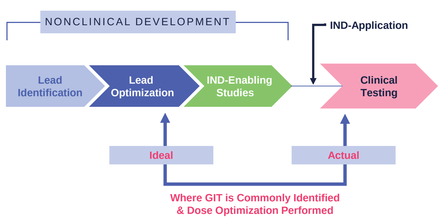
Ashley A. Spreen[1], Lauren Boone[1], Yuli Wang[2], Christopher E. Sims[2], Nancy L. Allbritton[2], Shimeng Liu[3], William R. Thelin[1], Elizabeth M. Boazak[1]

[1]Altis Biosystems, Durham, NC, [2]Department of Bioengineering, University of Washington, Seattle, Washington, [3]AstraZeneca Pharmaceuticals, R&D, Waltham, MA

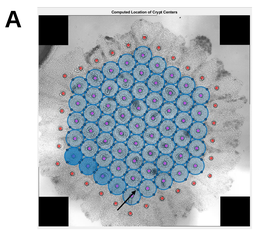
Abstract 336

Early Detection of GI Toxicity

Drug toxicity to the GI tract can heavily affect clinical trial process and outcomes. A mechanistic understanding of GI toxicity, along with preclinical data supporting precise dosing, could enhance clinical trial efficiency. Currently available in vitro GI models lack long-term dosing capabilities. We aimed to create an in vitro model that mimics gut turnover processes (proliferation, differentiation, and migration) to enable more physiologically relevant compound testing.



Development of Custom Analysis Tools



Red circles indicate the computed crypt unit centers. Blue circles indicate the crypt unit ROIs that are used for quantification.

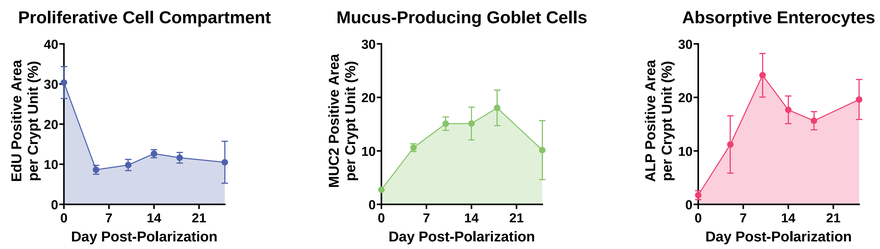
Paired fluorescent image for brightfield image shown in (A).

Each datapoint represents an individual crypt unit with min to max distribution

Custom image analysis enables quantitation of fluorescent staining for each crypt unit. Standard staining panel includes DAPI (total cells), EdU (proliferative cells), MUC2 (goblet cells), and ALP (mature enterocytes).

Quantitation of Cell Population Over Time

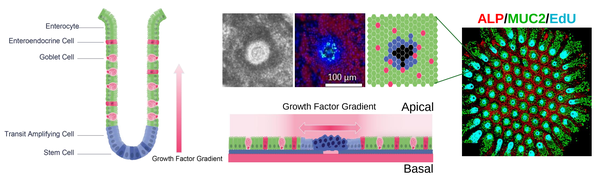
Steady maintenance of proliferative cells, goblet cells and absorptive enterocytes over 21 days



Figures depict mean ± SEM for n = 3 wells per time point. Each data point represents the average percent positive area per crypt unit for the 61 interior crypt units.

RepliGut® 2DCrypt™ Overview

Long term in vitro GI model developed by mimicking in vivo growth factor gradient



Engineered cell culture inserts consist of microhole arrays in an impermeable substrate covered by a hydrogel scaffold. Apical application of maturation media and basal application of stem-cell maintenance media generate a growth factor gradient, promoting tissue self-organization. The in vitro crypt units include a stem cell niche/proliferative zone (EdU+) surrounded by cells differentiating into absorptive and secretory lineages (ALP+/MUC2+).

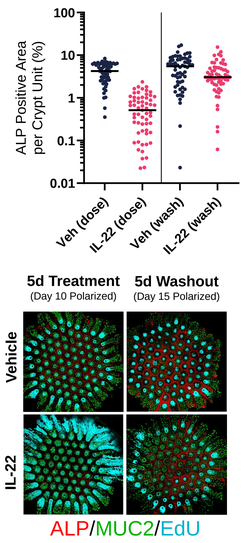
Platform Highlights

The RepliGut® 2D Crypt™ model:

- Replicates the spatial organization and self-sustaining nature of intestinal crypts
- Permits repeat and long-term dosing to enable assessment of both adverse and therapeutic responses to gut-targeted interventions
- Distinguishes between pro-proliferative and cytostatic effects of test compounds

RepliGut® 2D Crypt™ Applications: Cell Population Shift Case Studies

RepliGut® 2D Crypt™ detects reversible compound effects

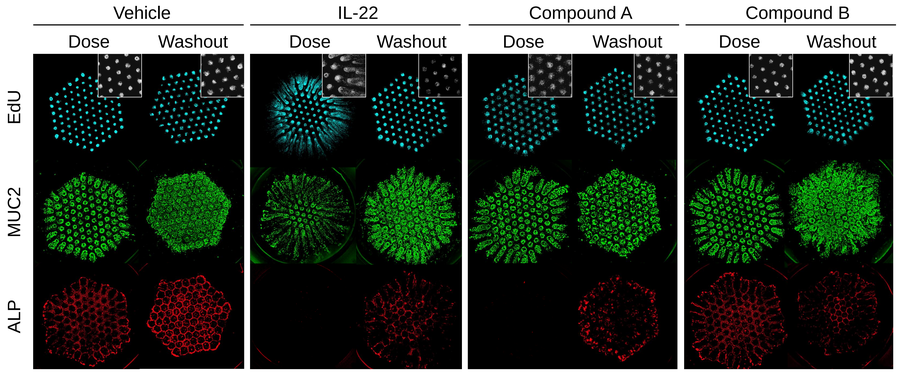


In vivo, over-production of IL-22 causes hyperproliferation, crypt lengthening, and loss of differentiation. IL-22 treatment for 5 days in RepliGut® 2D Crypt™ resulted in a 7-fold reduction in ALP+ area per crypt, as well as a marked expansion of proliferation (EdU+ Area). Effects were largely reversed following a 5-day washout (Wash).

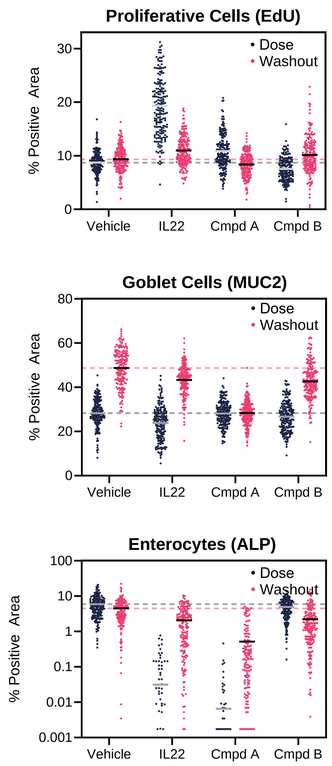
Evaluation of AstraZeneca preclinical compounds

Compounds were added to the apical and basal compartments daily for 7 days. Compound A, known to be pro-proliferative, increased EdU+ area, while decreasing ALP+ area relative to vehicle. Compound B, with known cytostatic effects, decreased EdU+ area, with minimal impact on other cell compartments.

IL-22 and compound A effects were significantly reversed after a 7-day washout (Washout). Compound B treatment resulted in decreased ALP+ area after the washout, possibly due to delayed onset of cell differentiation



Top: Representative single channel micrographs of one well from each treatment group. Insets show EdU staining of crypt units along the edge of the array.
Right: Quantification of n = 3 wells per treatment, with 61 crypt units per well. Each data point represents one interior crypt unit. Horizontal bars indicate the group means. Pink and blue dashed lines indicate the dose or washout vehicle mean, respectively.



Visit altisbiosystems.com

Contact:
Liz Boazak, PhD
liz@altisbiosystems.com