Primary Human Small Intestine Monolayers For Assessing Drug-Induced Intestinal Liabilities *In Vitro*

#3850/ P340

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BACKGROUND

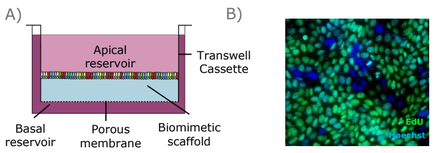
Drug-induced gastrointestinal (GI) complications are the most frequent adverse events reported in clinical trials. At present, late and expensive *in vivo* animal studies remain the cornerstone of preclinical GI safety evaluation. Stem cell-based *in vitro* assays hold the potential to improve early candidate compound selection and mechanistic investigations, helping to accelerate programs and reducing the number of animals used.

OBJECTIVE

To explore if a primary human small intestine (SI) monolayer cell culture system (RepliGut® Planar Platform) could **distinguish between compounds with different potencies for the JNJ epigenetic target X with a known *in vivo* GI liability**, including loss of expression of the intestinal stem cell gene *OLFM4*. To evaluate if the assay was adequate for this intended use, we first **tested a limited set of reference compounds including drugs with a high (> 50%) and a low (0-4%) incidence of diarrhea in the clinic.**

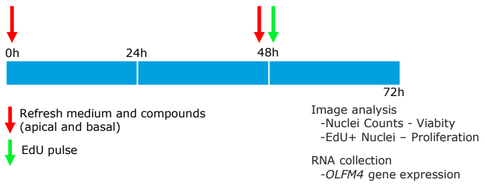
METHODS

Figure 1. Schematic representation of Altis RepliGut® Planar Platform (A). Confocal microscope image of a jejunal monolayer showing EdU+ proliferative cells (B).



Human jejunal epithelial monolayers in cell expansion phase contain a majority of proliferative 5-Ethynyl-2'-deoxyuridine (EdU)-positive cells (stem and progenitor cells).

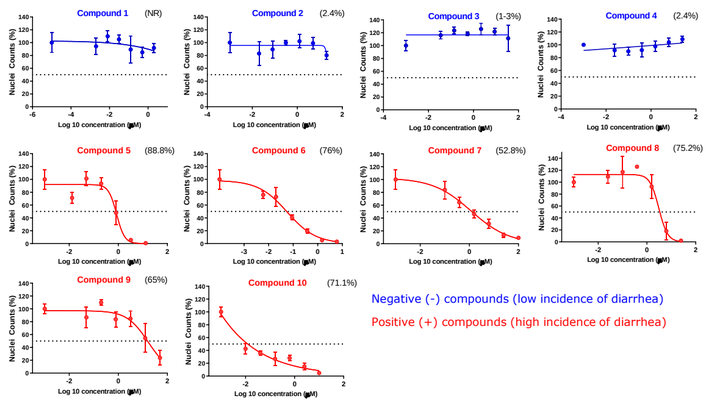
Figure 2. Overview of the experimental design



Detailed methods for image analysis can be found on Ref. 1. Monolayers were incubated with reference and JNJ compounds at *in vitro* concentrations up to 100-fold the clinical C_{max} (Ref.2) and 50 µM, respectively.

RESULTS & DISCUSSION

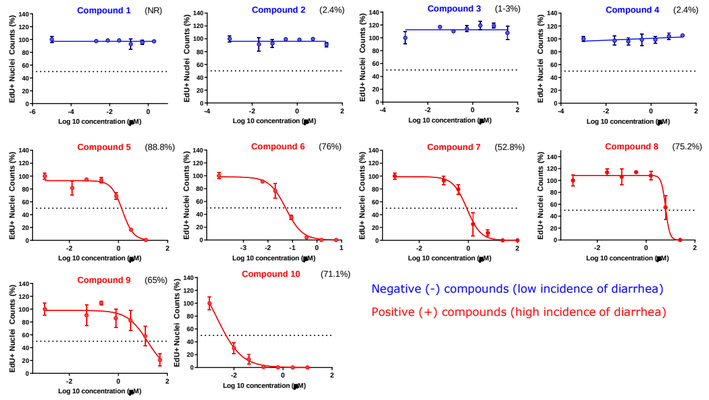
Figure 3. Viability endpoint distinguishes (+) and (-) reference compounds in Altis RepliGut®



Incidence of diarrhea in the clinic is shown between (brackets). Dots and bars represent mean and SD, respectively. Horizontal dashed line shows 50% reduction. NR, not reported

Viability of Altis RepliGut® was quantified as nuclei count (Ref. 1). Following a 72-hour incubation, all 6 (+) but none of the 4 (-) compounds reduced viability and proliferation of jejunal monolayers more than 50% of DMSO controls (Figures 3 and 4).

Figure 4. Proliferation endpoint distinguishes (+) and (-) reference compounds in Altis RepliGut®



Incidence of diarrhea in the clinic is shown between (brackets). Dots and bars represent mean and SD, respectively. Horizontal dashed line shows 50% reduction. NR, not reported

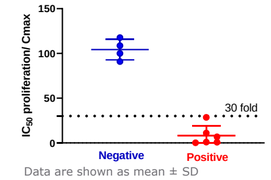
Proliferation was quantified as % EdU+ nuclei (Ref. 1). Positive drugs exhibited a proliferation IC50 to clinical Cmax ratio of ≤30 (Figure 5; Ref. 3).

Figure 5. Proliferation inhibition (IC_{50}) of (+) and (−) reference compounds normalized to clinical exposure (A). IC_{50}/C_{max} ratios of (+) and (−) reference compounds (B)

A)

	IC_{50} (µM)	C_{max} (µM)	IC_{50}/C_{max}
Compound 1	>2	0.02	100
Compound 2	>20	0.17	117.65
Compound 3	>35	0.35	100
Compound 4	>25	0.23	108.7
Compound 5	1.44	0.13	11.08
Compound 6	0.05	0.06	0.83
Compound 7	0.87	1.02	0.85
Compound 8	6.28	0.22	28.55
Compound 9	15.94	2.33	6.84
Compound 10	<0.01	0.07	0.14

B)



Data are shown as mean ± SD

Figure 6. Confocal microscope image confirming expression of JNJ target X in jejunal monolayers on day 2. Nuclei staining (A), JNJ target staining (B) and overlay (C)

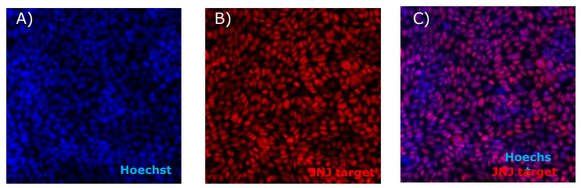
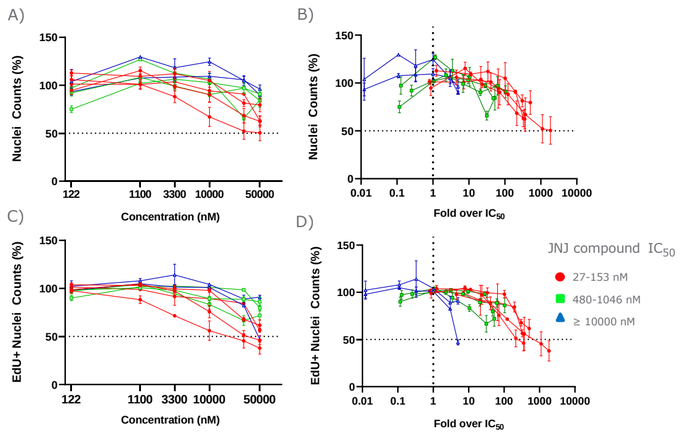


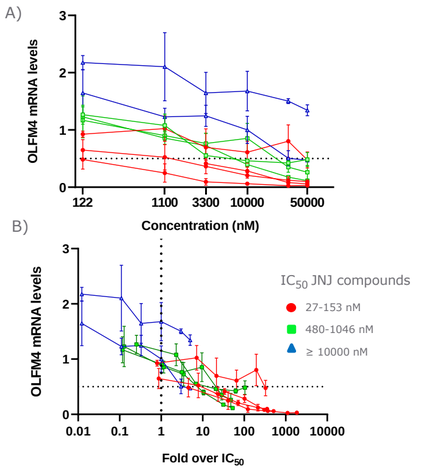
Figure 7. Viability (A,B) and proliferation (C,D) in jejunal monolayers after dosing with JNJ compounds inhibiting target X.



Results are plotted against nominal concentrations (A, C) or against the ratio of nominal concentrations to the compound IC50 (B,D). Dots and bars represent mean and SD, respectively. Horizontal dashed line shows 50% reduction. Vertical dashed line shows Concentration/IC_{50} ratio=1.

Viability and proliferation readouts were not sensitive enough to assess the impact of inhibition of JNJ target X in human jejunal monolayers (Figure 7) .

Figure 8. JNJ compounds inhibiting target X induce a dose-dependent decrease in *OLFM4* expression.



Results are plotted against nominal concentrations (A) or against the ratio of nominal concentrations to the compound IC50 (B). Dots and bars represent mean and SD, respectively. Horizontal dashed line shows 50% reduction. Vertical dashed line shows Concentration/IC_{50} ratio=1.

At the same concentration, the reduction of *OLFM4* was more pronounced with increasing potency for the JNJ target X (Figure 8).

CONCLUSION

- Altis RepliGut® distinguishes (+) and (-) reference compounds using viability and EdU incorporation readouts.
- Altis RepliGut® captures differences in potency for JNJ target X between different inhibitors based on *OLFM4* expression.
- These data support the use of stem cell-based models for predicting and mechanistically evaluating novel oncology drugs with non-cytotoxic mechanisms of action, therefore contributing to more informed selection of compounds prior to *in vivo* studies.
- Depending on the target being evaluated, other readouts besides traditional viability and proliferation measurements should be considered.

REFERENCES

1. ACS Infect Dis. 2018 Jan 12; 4(1): 46–52
2. Toxicol Sci. 2012 Mar;126(1):114-27
3. Toxicol In Vitro. 2020 Oct;68:104928

Conflict of interest:
Jardi F., Taylor D., Kanerva J., and Yanochko-Hoffman G.M. are employees of Janssen R&D company.
McQueen B. and Zwarycz B are employees of Altis Biosystems.

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