Evaluation of 3D human iPSC derived intestinal organoids as a platform for EV-A71 antiviral drug discovery

Fatma Masmoudi[1,2], Nanci Santos-Ferreira[3], Xin-Xuan Lin[1], Susanna Poghosyan[1], Sara Compte-Sancerni[1], Dasja Pajkrt[4], Katja Wolthers[2], Jeroen DeGroot[1], Maria L.H. Vlaming[1], Jan Stallen[1], Joana Rocha-Pereira[3] and Ludovico Buti[1]

1 Charles River Laboratories, Leiden, the Netherlands 2 Amsterdam UMC, University of Amsterdam, Amsterdam Institute for Infection and Immunity, Department of Medical Microbiology, OrganoVIR Labs, Amsterdam, the Netherlands 3 KU Leuven-Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium 4 Amsterdam UMC, University of Amsterdam, Department of Pediatric Infectious Disease, OrganoVIR Labs, Amsterdam, the Netherlands

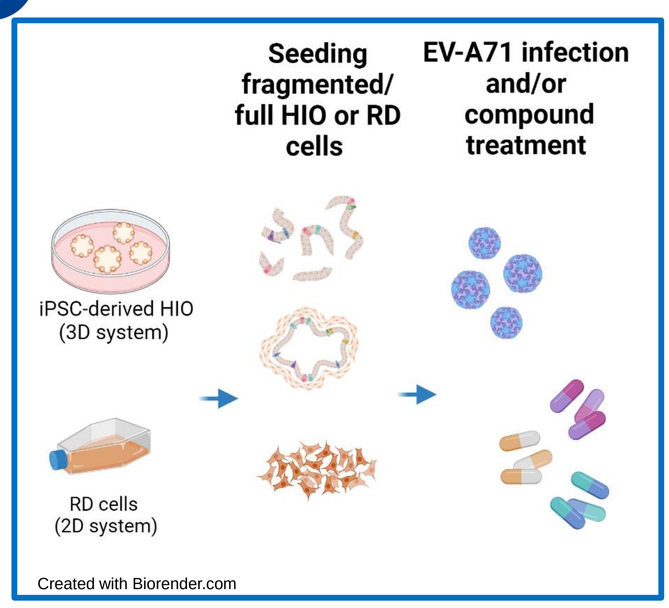
1 Overview

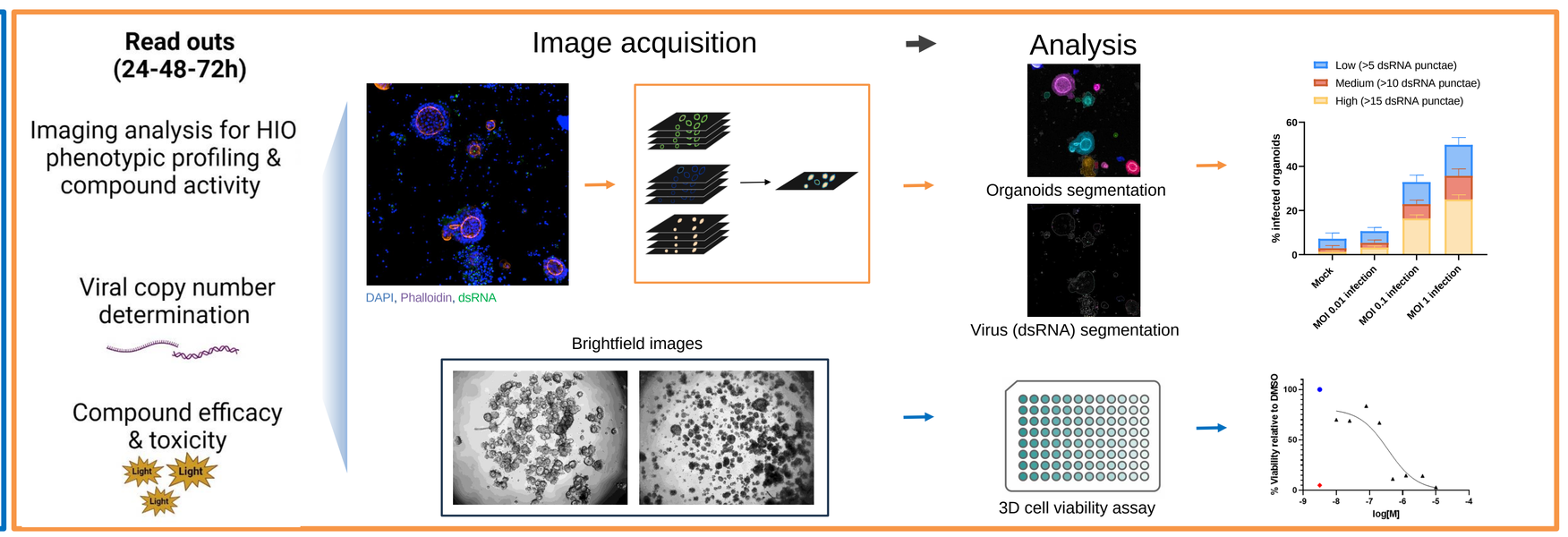
- Identification of specific antiviral treatments are challenging due to a lack of suitable physiologically relevant model systems and strategies
- Complex three-dimensional human multiple cell type models (organoids) are being explored to study antiviral effects in early drug discovery
- We have utilized iPSC derived human intestinal organoids to establish a platform to test effects of antiviral drug treatment

2 Introduction

- EV-A71 is mainly transmitted through the respiratory and gastrointestinal tract, causing a range of mild to severe symptoms in children
- There is currently no antiviral agent known to be effective in treating EV-A71 infection
- iPSC-derived Human Intestinal Organoids (HIOs) were used to model EV-A71 infection and replication and compared to a conventional cell line (RD cells)
- Three reference compounds, with various modes-of-actions and different safety profiles were tested in this pilot study

2 Methods





4 Results

Fully differentiated HIOs are amenable for EV-A71 infection and replication which causes cytopathic effect (CPE)

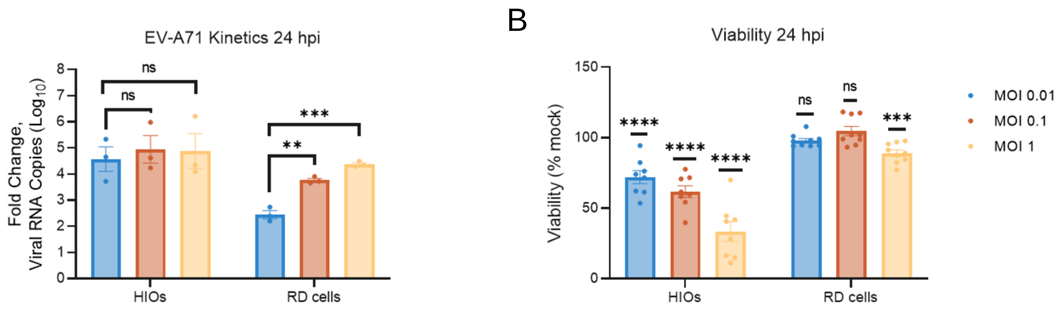


Figure 1. HIOs or RD cells infected with EV-A71 at different Multiplicity Of Infections (MOIs) and analysis of replication kinetics (A) and viability (B) at 24hr post infection (pi). Data are SEM of *n*=3, ** indicates p < 0.01, *** indicates p < 0.001, and **** indicates p < 0.0001; ns indicates not significant

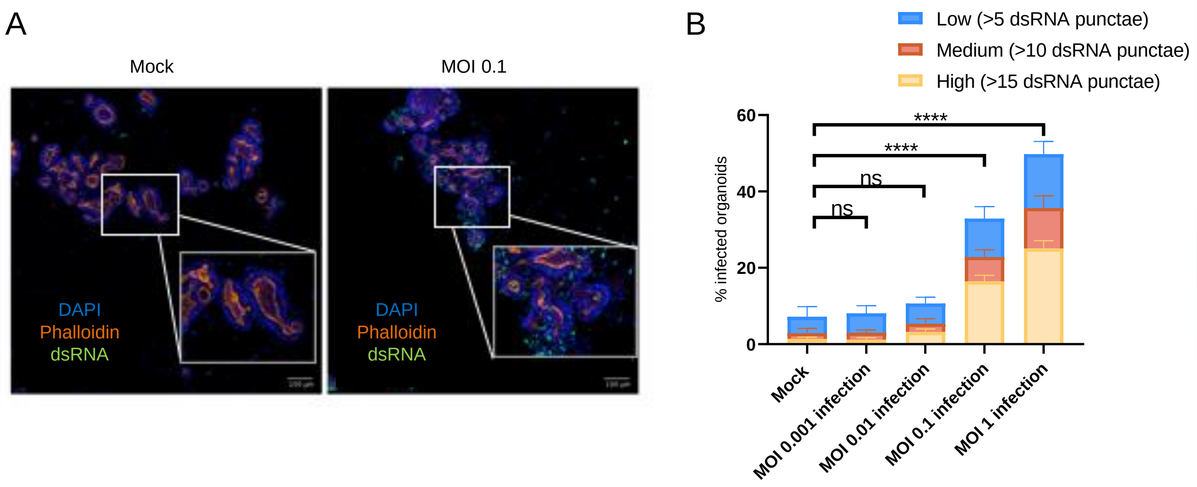


Figure 2. Immunofluorescence of HIOs infected with EV-A71 (0.1 MOI). Staining for nuclei (DAPI, blue), actin (Phalloidin, orange) and EV-A71 (dsRNA, green) (A). High content analysis of HIOs infected with EV-A71 at different MOIs and stained as in panel (A). Percentage of infected organoids were quantified based on the number of dsRNA staining/organoid. Data are SEM of *n*=3, **** indicates p < 0.0001; ns indicates non significant (B).

Concentration-dependent antiviral activity of compounds determined by CPE

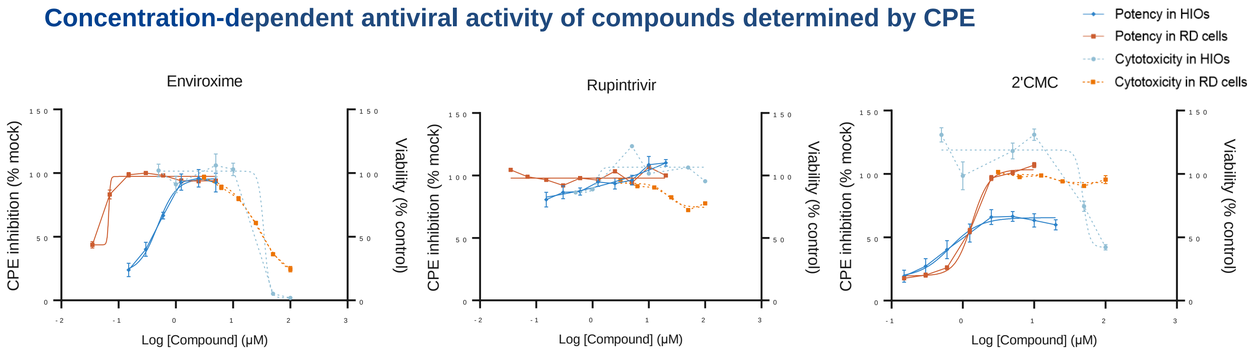


Figure 3. Antiviral activity determined by CPE inhibition (at non-toxic compound concentration, continuous lines) and viability determined by cytotoxicity of the compounds (dotted lines), in HIOs (*blue*) and RD cells (*red*) at 72 hpi.

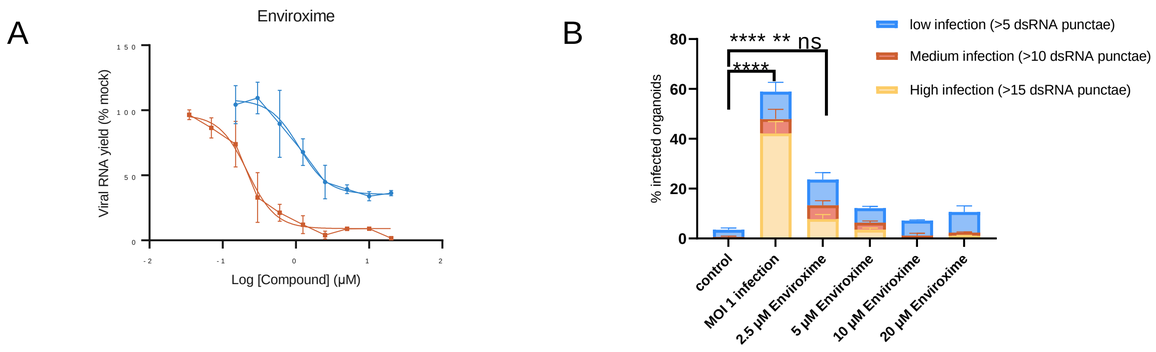


Figure 4. Antiviral activity of Enviroxime determined by measuring viral RNA yield in HIOs and RD cells at 72 hpi (A) and by high content analysis of HIOs infected with EV-A71. Data are SEM of *n*=3, **** indicates p < 0.0001; ** p < 0.01 and ns indicates non significant (B)

Compounds	Enviroxime			Rupintrivir			2'CMC		
	CC_{50} (µM)	EC_{50} (µM)	SI*	CC_{50} (µM)	EC_{50} (µM)	SI	CC_{50} (µM)	EC_{50} (µM)	SI
HIOs	24 ± 7	0.4 ± 0.2	60	>100	<0.15	>667	48 ± 1	1.0 ± 0.3	48
		1.4 ± 0.3	17		1.7 ± 0.4	>59		1.5 ± 0.3	32
RD cells	28 ± 2	0.06 ± 0.001	467	>100	<0.035	>2857	>100	1.3 ± 0.04	>76
		0.2 ± 0.04	140		<0.035	>2857		2 ± 0.4	>50

Table 1. CC_{50}, EC_{50}, and selectivity indices of enviroxime, rupintrivir, and 2'CMC in HIOs and RD cells.

*Selectivity Index (SI) = CC_{50}/EC_{50}. EC_{50} values in black were based on CPE inhibition data, EC_{50} values in blue were based on RNA yield data. Values are expressed as mean ± SEM.

5 Conclusions

- These results indicate that more complex and physiologically relevant cell models (HIO) can help improve and re-fine pre-clinical candidate selection, when compared to more simple 2D conventional cell models as increased viral yields, increased sensitivity to compound treatment and decreased antiviral activities are observed for the HIOs. Our data is in line with previous reported pre-clinical safety issues of these compounds
- A high-content image analysis to profile viability and compound's efficacy in EV-A71-infected organoids has now been established
- Multiple iPSC-derived organoid models will provide series of powerful and efficient platforms for studying effects of drugs in several human diseases