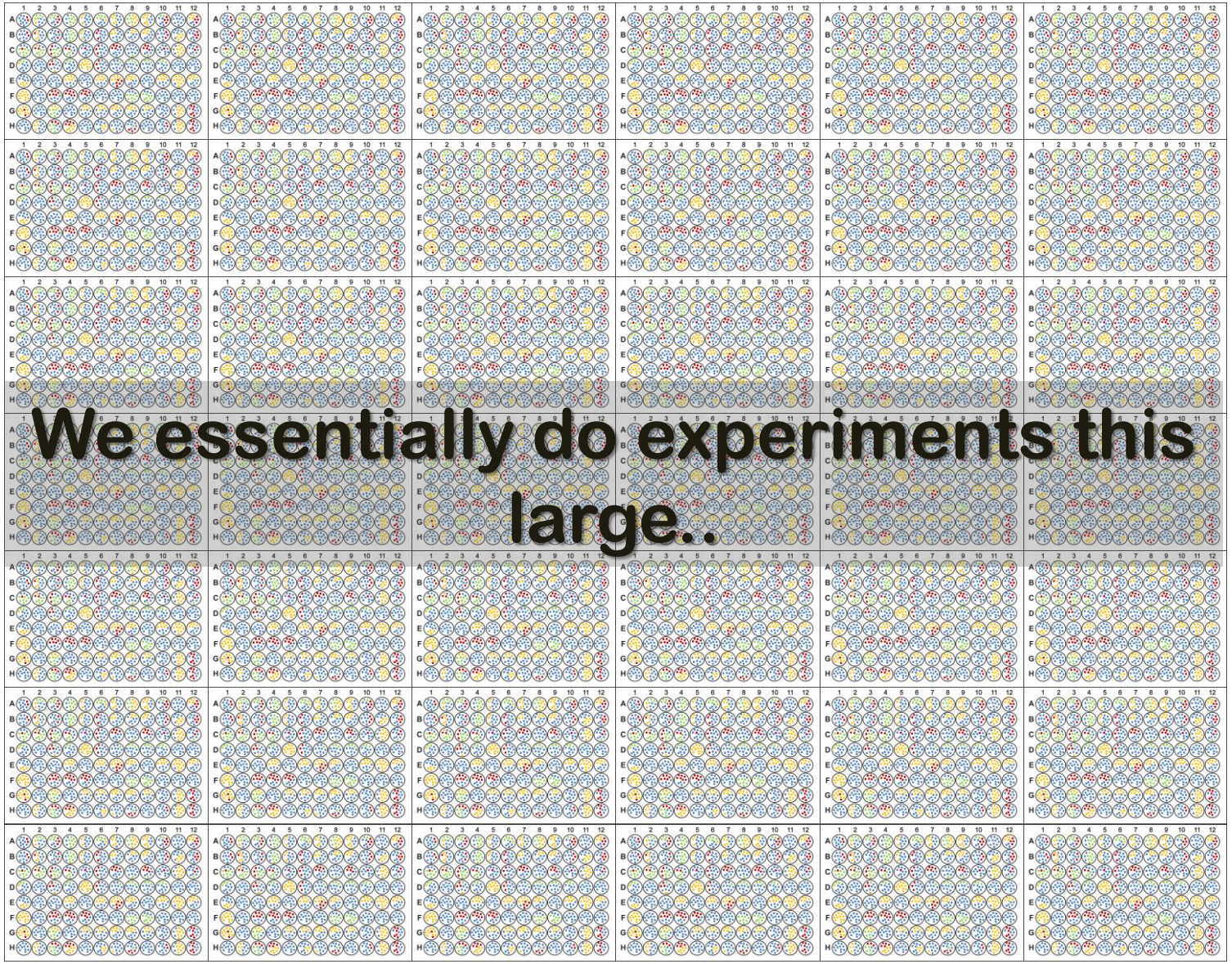
Exploration of HD-DoE methods to identify combinatorial effects of anti-viral drugs
- SARS-CoV2 -

Project was done together with the ERINHA Node at KUL, Belgium

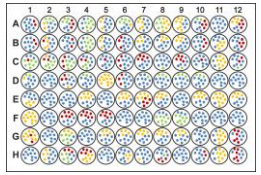
Our tool is HD-DoE technology – Exploring Combinations

HD-DoE: high-dimensional design-of-experiments



We essentially do experiments this large..

COMPRESSED DESIGN



..at a very reduced cost

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HD-DoE applications by Trailhead Biosystems

Demonstration of proof-of-concept for Viral interference (COVID-19)

1 Regenerative medicine

2 Viral interference

3 Precision Medicine in Cancer

Development of combinatorial formulation against SARS-CoV2

Performance of anti-viral screen (BSL4 robotics) with KU-Leuven CAPS-IT

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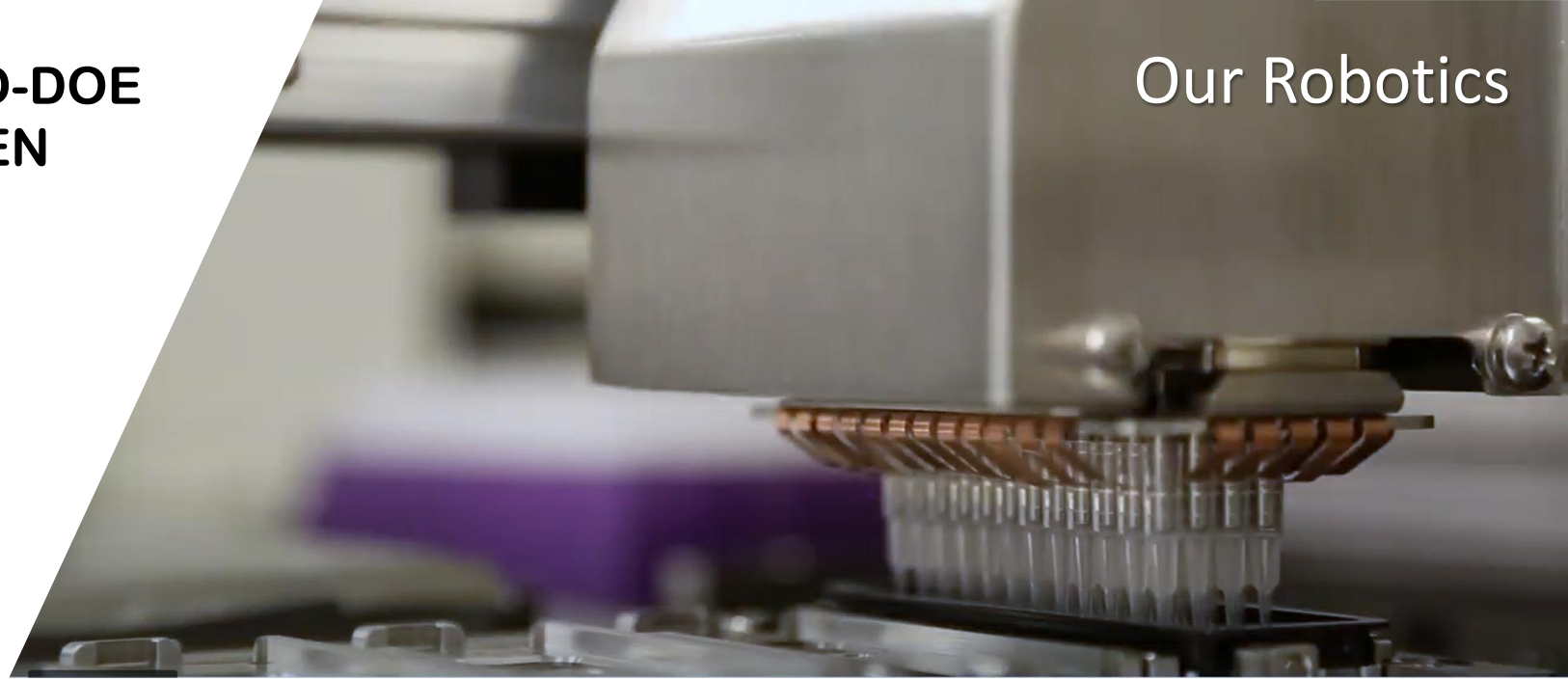
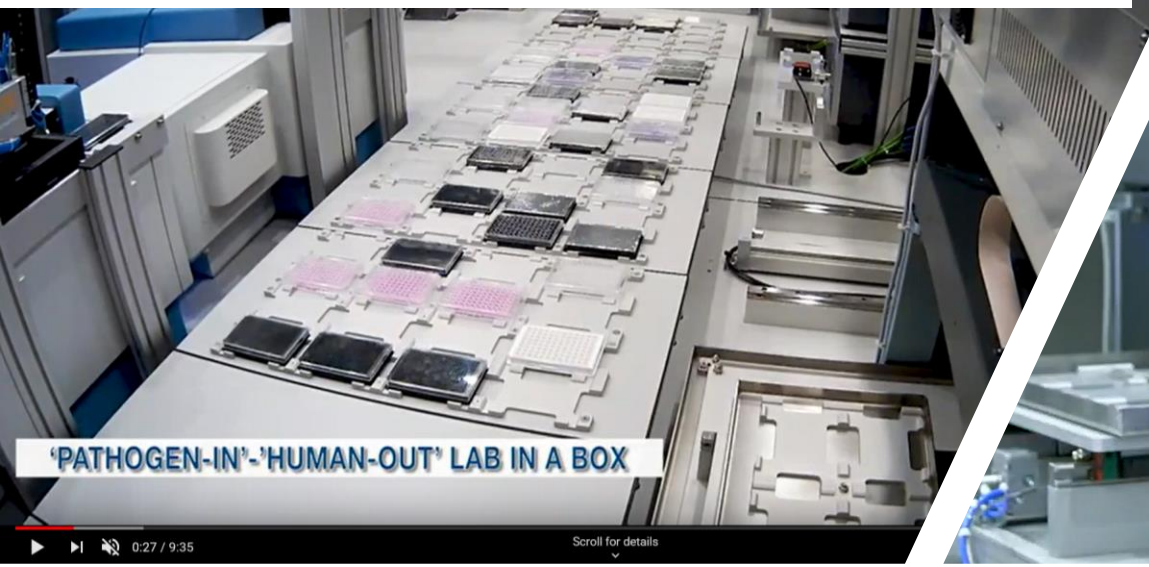
Manufacturing and Shipping of HD-DOE ANTIVIRAL COMBINATION SCREEN

Plate Libraries made at Trailhead Biosystems (April to May, 2020)



KU Leuven: Testing HD-DoE library in the worlds' only robotic BSL4 facility



KU LEUVEN

KU Leuven, Belgium: CAPS-IT system

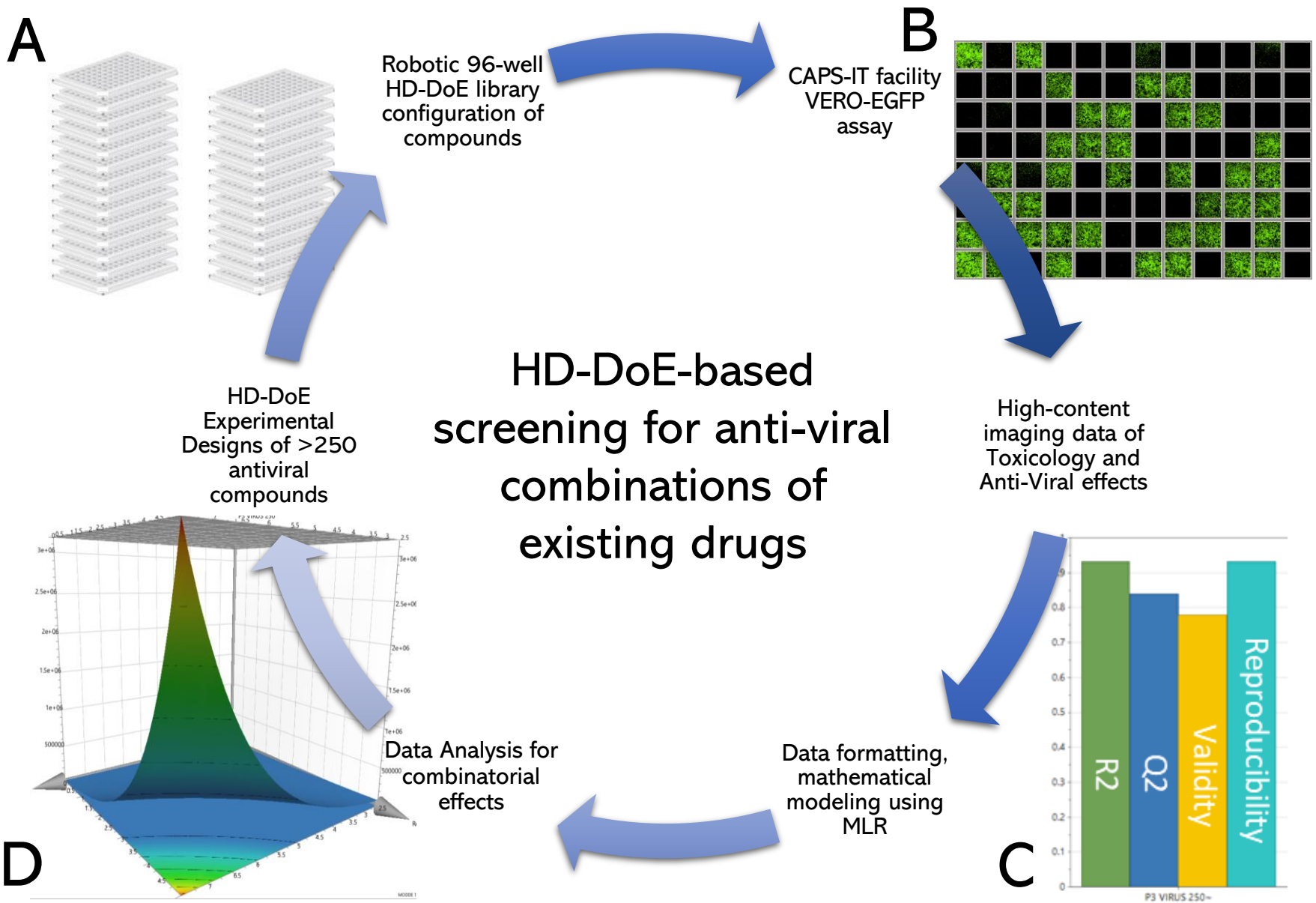
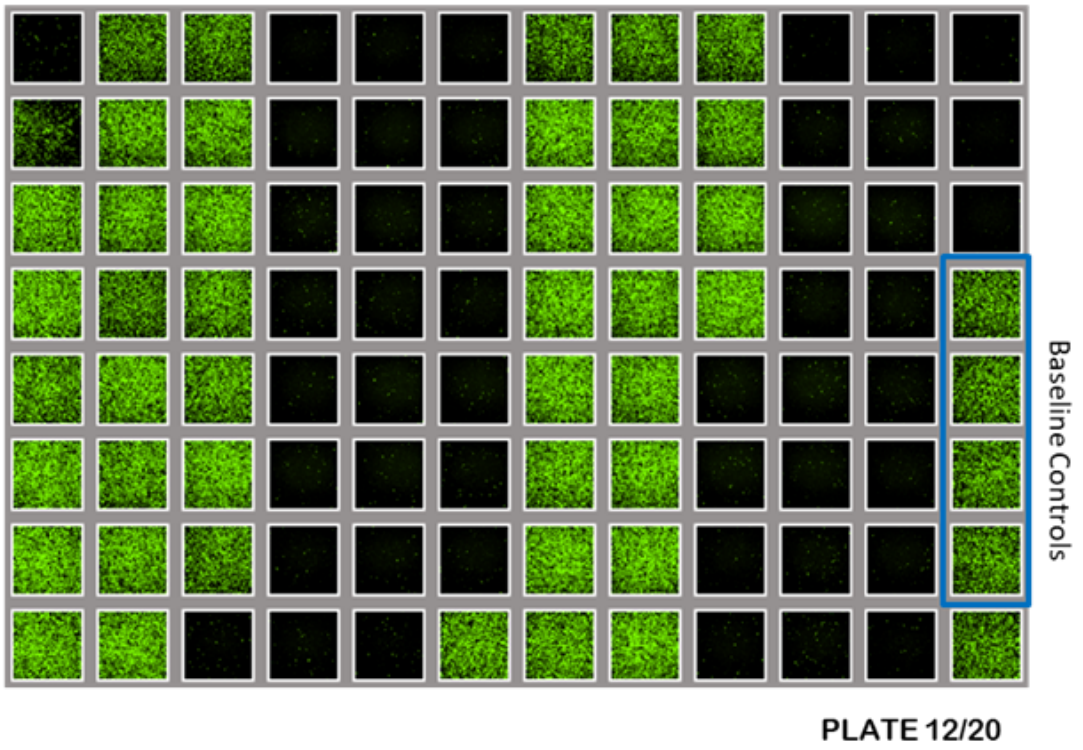

A
Robotic 96-well HD-DoE library configuration of compounds
B
CAPS-IT facility VERO-EGFP assay
High-content imaging data of Toxicology and Anti-Viral effects
C
R2
Q2
Validity
Reproducibility
P3 VIRUS 250~
Data formatting, mathematical modeling using MLR
Data Analysis for combinatorial effects
D
HD-DoE Experimental Designs of >250 antiviral compounds
HD-DoE-based screening for anti-viral combinations of existing drugs

Figure 1

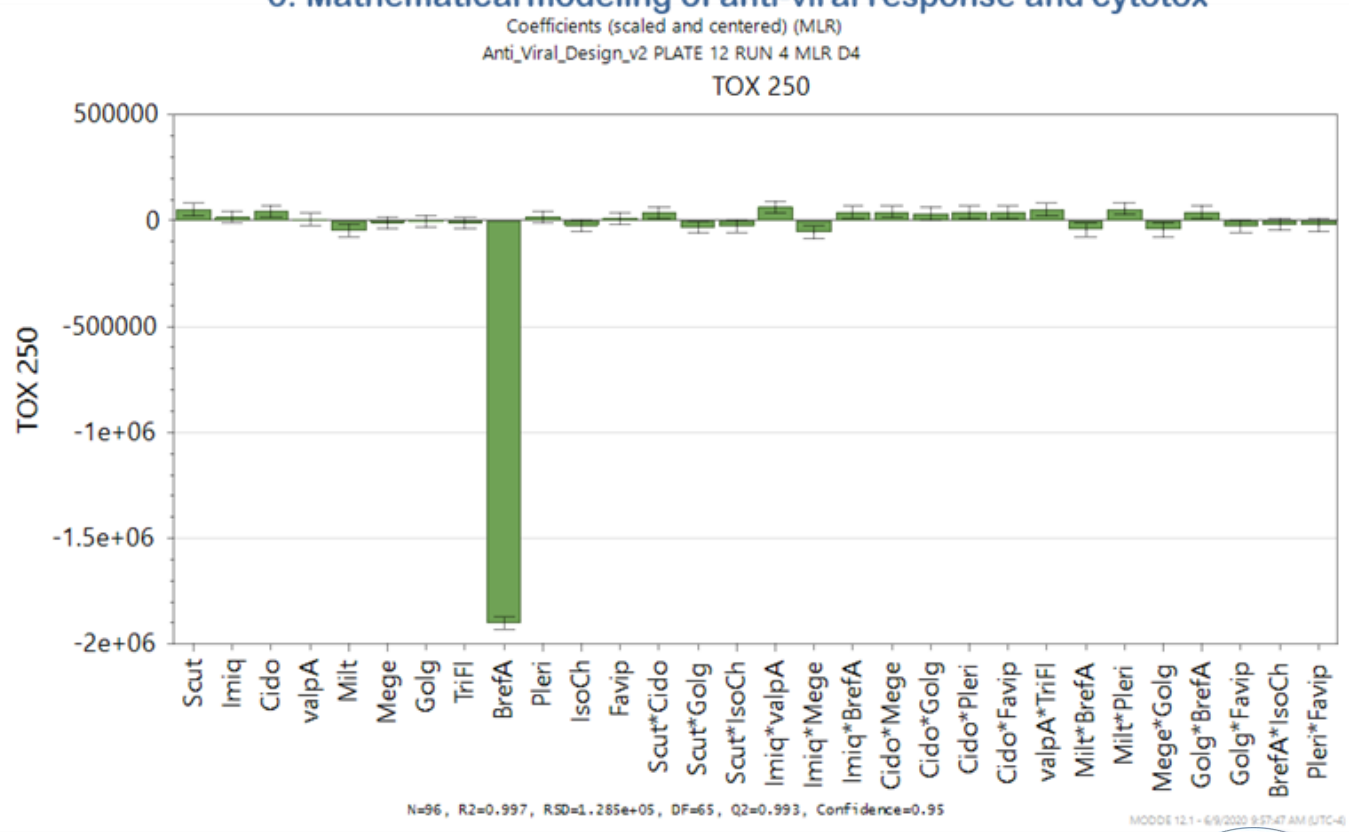
TRAILHEAD DoE plates > CAPS IT Data > TRAILHEAD ANALYSIS

1. Our combination Input plate

2. CAPS IT scan data



3. Mathematical modeling of anti-viral response and cytotox



Example Toxicology screen

R2 = 0.997, Q2 = 0.993

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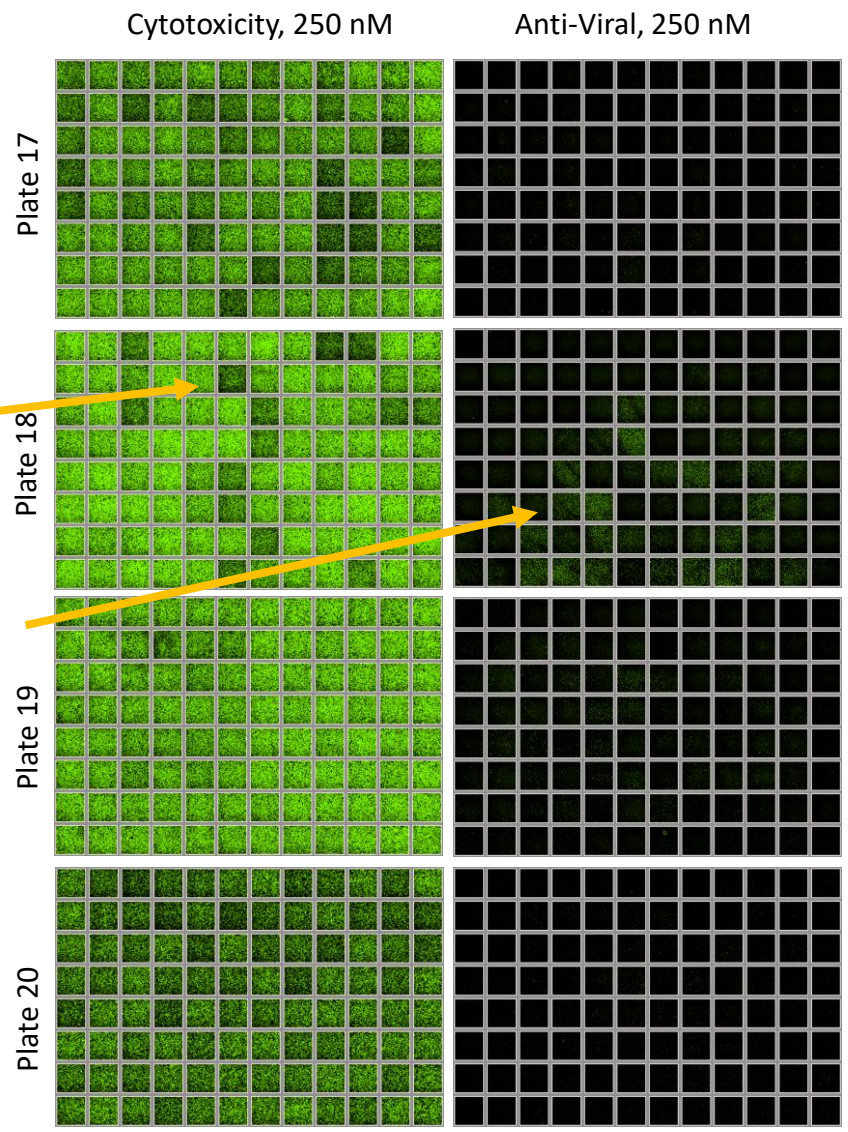
A Typical Assay Read out – Plate 17-20



Weak cytotoxicity

Some combinatorial effects

Tested Compounds

Cytotoxic compounds

Phenytoin	BLZ945
Artesunate	Germacrone
SC75741	epicatechin
ESI09	BIO-Acetoxime
Verdexinor	FTI 277
ML324	Favipiravir
Lycorine	P-ethanolamide
Spautin-1	Rimantadine
Oltipraz	N-acetylNeuramidic
N6 Methyladenosine	Pyridoxal 5-P
Tizoxanide	Anthraquinone
3-DeAza adenosine	OH-Chloroquine
Hinokitiol	2 picolinic acid
Cycloserine	L Lysine
Oxindole	3(4-oh-Phenyl) p-acid
Etidronate	Glucorone
DL-a-Lipoic acid	Azithromycin
Pentoxyfylline	OH-Chloroquine
Amantidine	DH-andrographolide
Thiamine	3OH-4meOHcinnamic
Vitexicarpin	Linsippril
Psoralen	Camostat
Geniposide	Rapamycin
Bergenin	Favipiravir

SARS-CoV2 Assay
(Vero-EGFP cells)

TOX 250 nM (no virus, drugs only)

ANTIVIRAL 250 nM (Drugs + SARS-CoV2)

PLATE 20

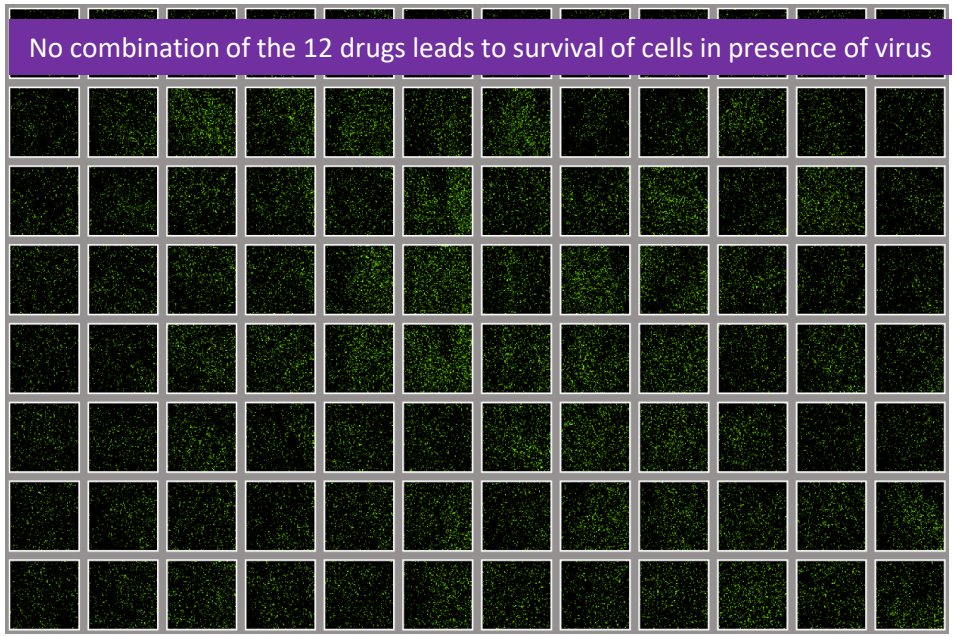
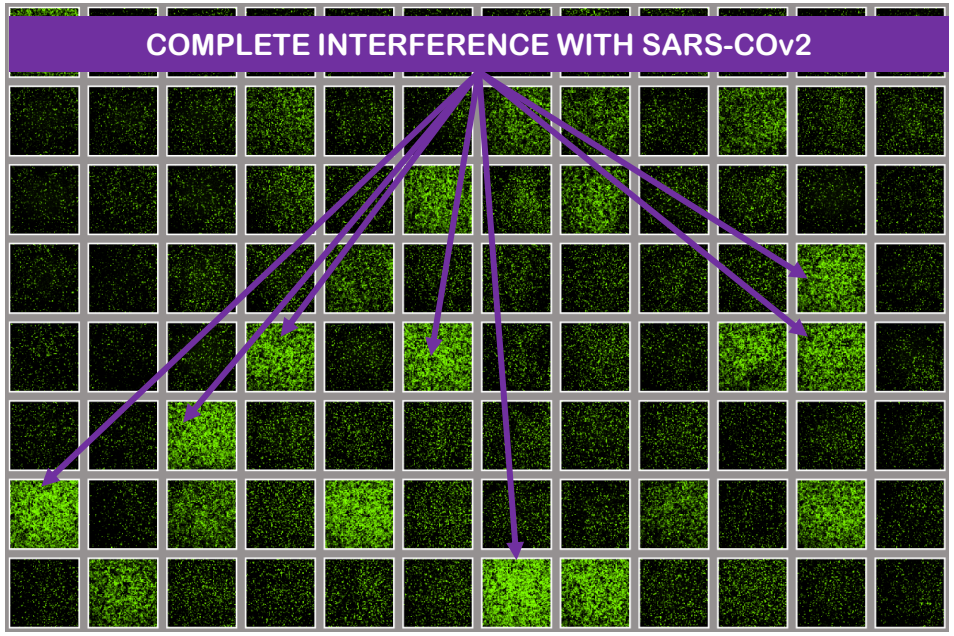


PLATE 22



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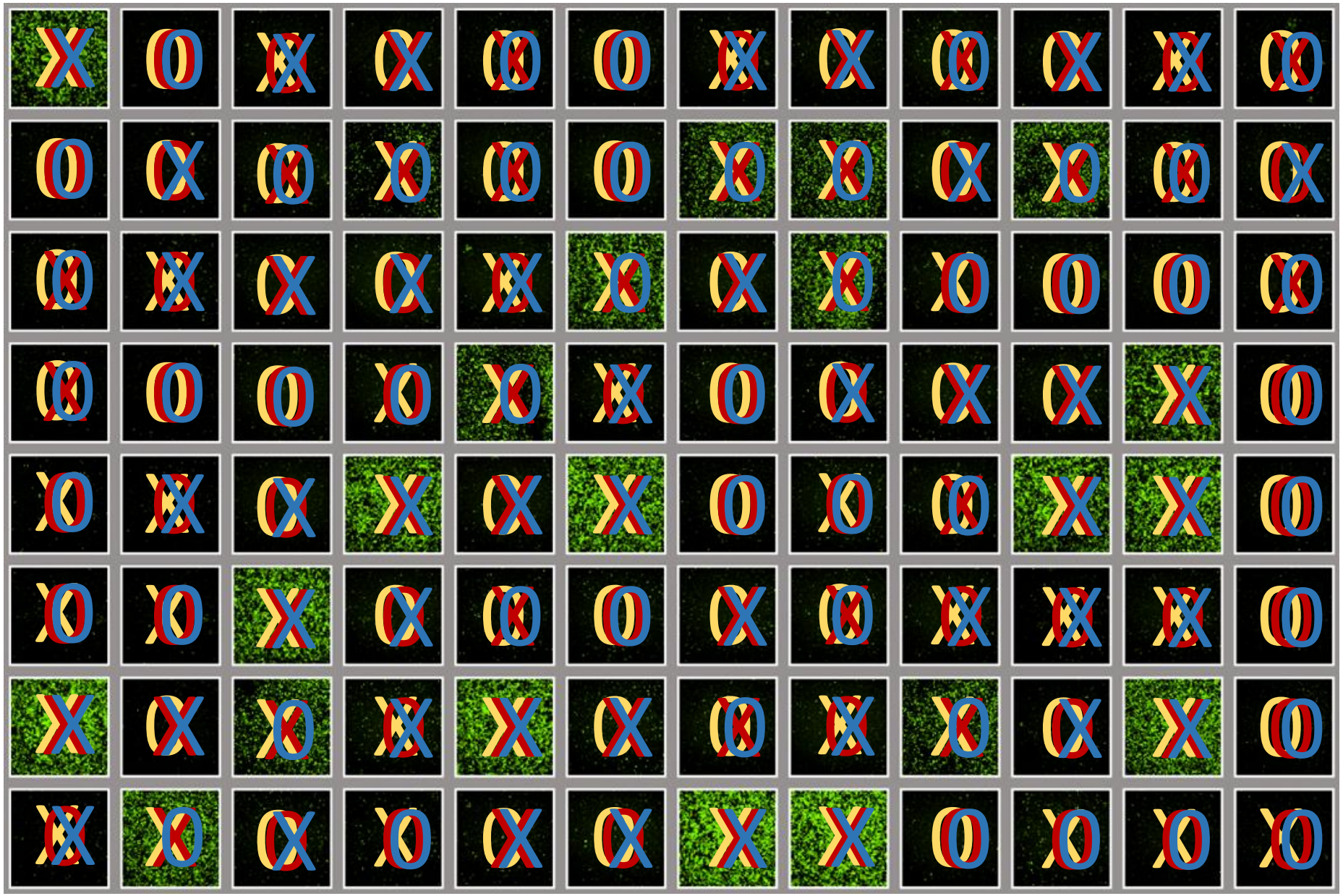
Trailhead Biosystems High-Dimensional Design of Experiments anti-viral combination screening

Remdesivir

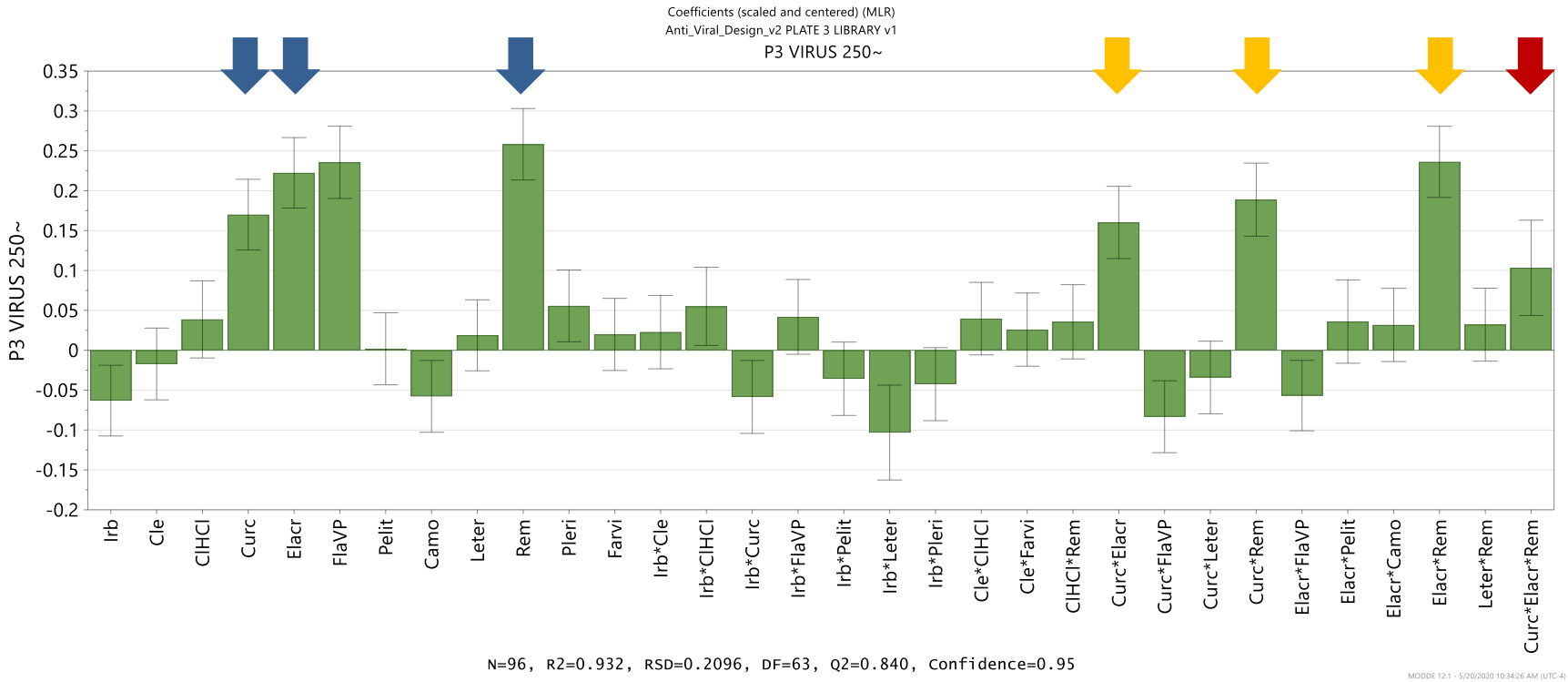
Elacridar

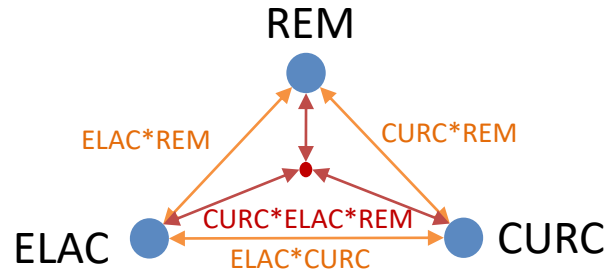
Curcumin



CI013820 (Trailhead plate 22 - AV 250, **250 nM of each drug** added to each well)

TRIPLE SYNERGISTIC ANTIVIRAL RESPONSE @ 250 nM





Primary Terms

1st order Interaction Terms

2nd order Interaction Term

PLATE 3/20

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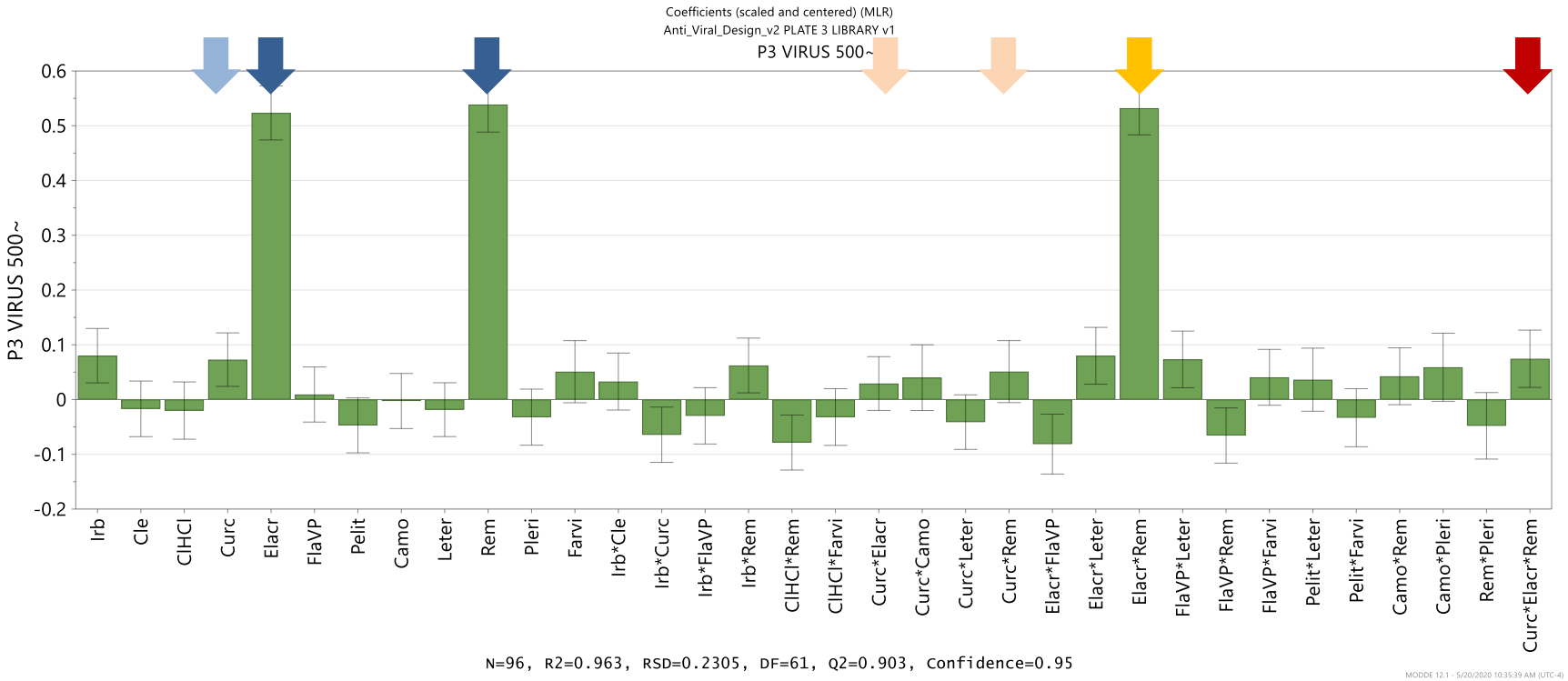
WEAKER TRIPLE SYNERGISTIC ANTIVIRAL RESPONSE @ 500 nM



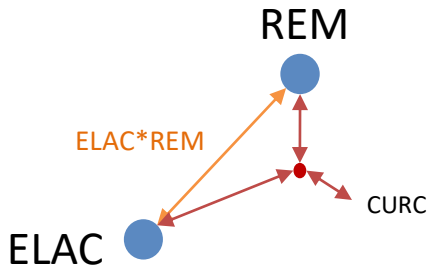


PLATE 3/20

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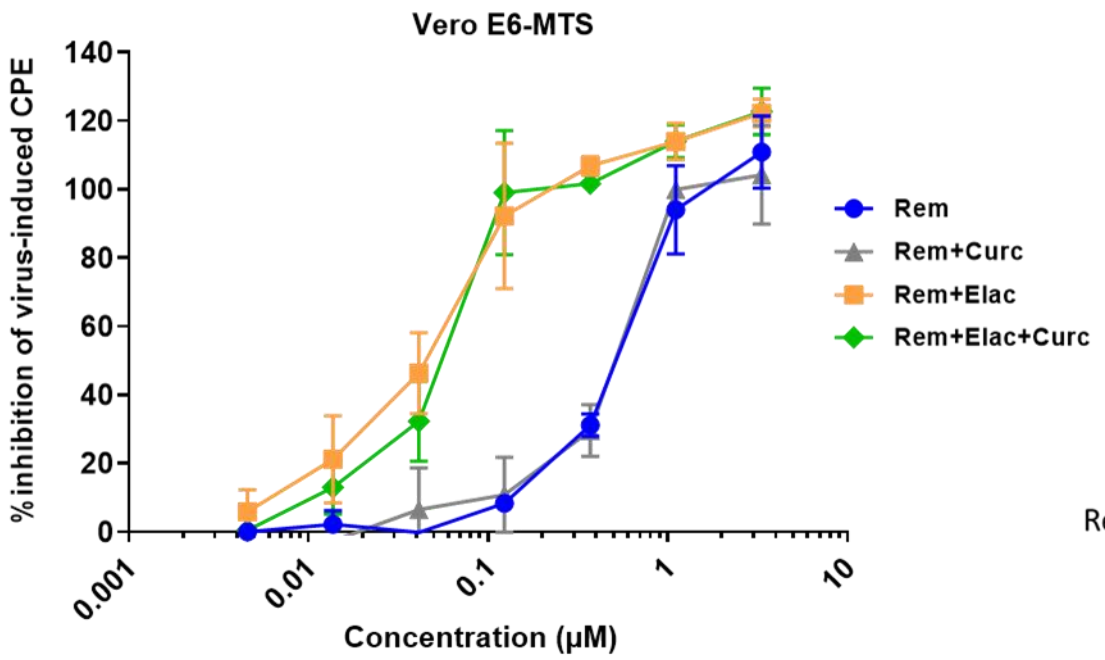
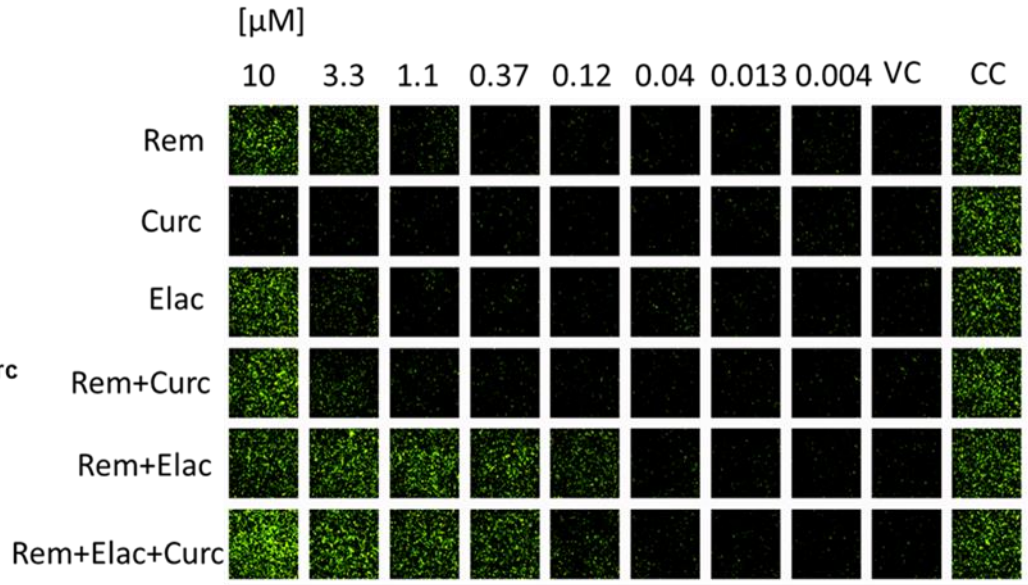
VALIDATION OF DOSE RESPONSE OF THE COMBINATION IN VERO6

A



B



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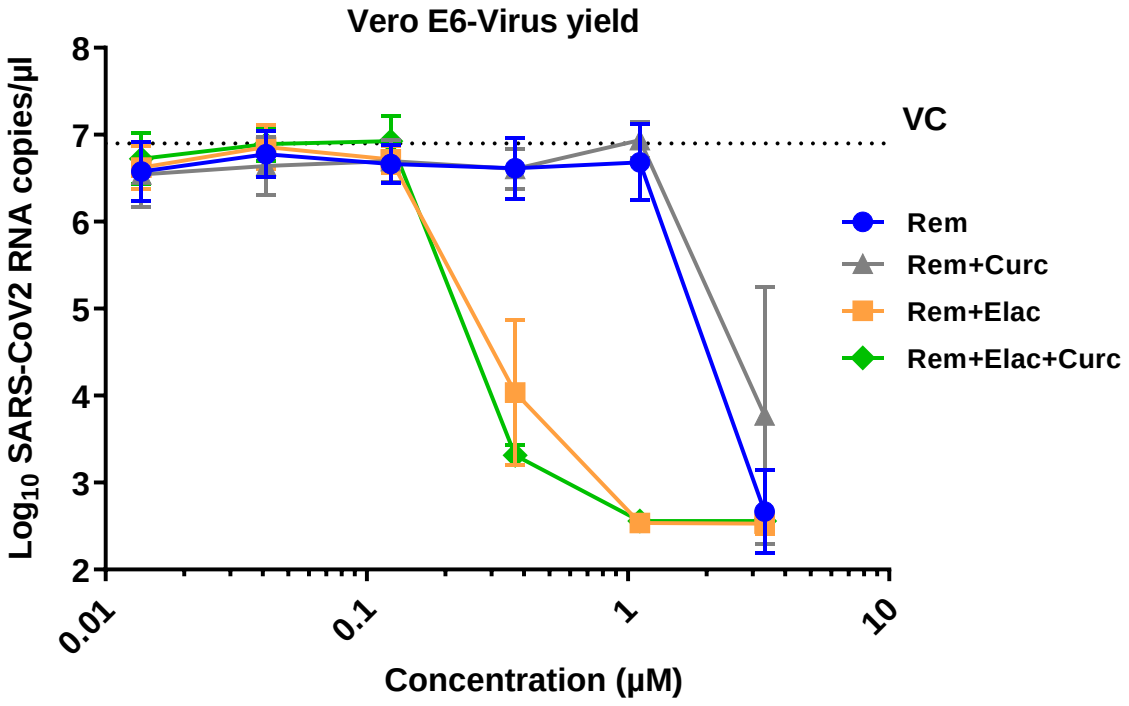
VALIDATION OF DOSE RESPONSE BASED ON VIRAL PRODUCTION



- 2h preincubation with compounds, MOI 0.1
- 20.000 cells/well, supernatant collected on 2 dpi

Compound(s)	Concentration (µM)		SI	*X-fold
	CC_{50} MTS	EC_{50}		
Rem	>10	2.8 ±0.3	>3.6	**1**
Curc	8	>8	nd	**/**
Elac	>10	>10	nd	**/**
Rem+Curc	7.1	>7.1	nd	**/**
Rem+Elac	>10	0.57 ±0.2	>17	**4.9**
Rem+Curc+Elac (REC)	7.5	0.39 ±0.1	19	**7.2**

EC_{50}, 50% effective concentration as determined by qRT-PCR
CC_{50}, 50% cytotoxic/cytostatic concentration
**X-fold= EC_{50} remdesivir/EC_{50} combo*
SI, selectivity index= CC_{50}/ EC_{50}
Data are mean values ± SD of at least two independent experiments.

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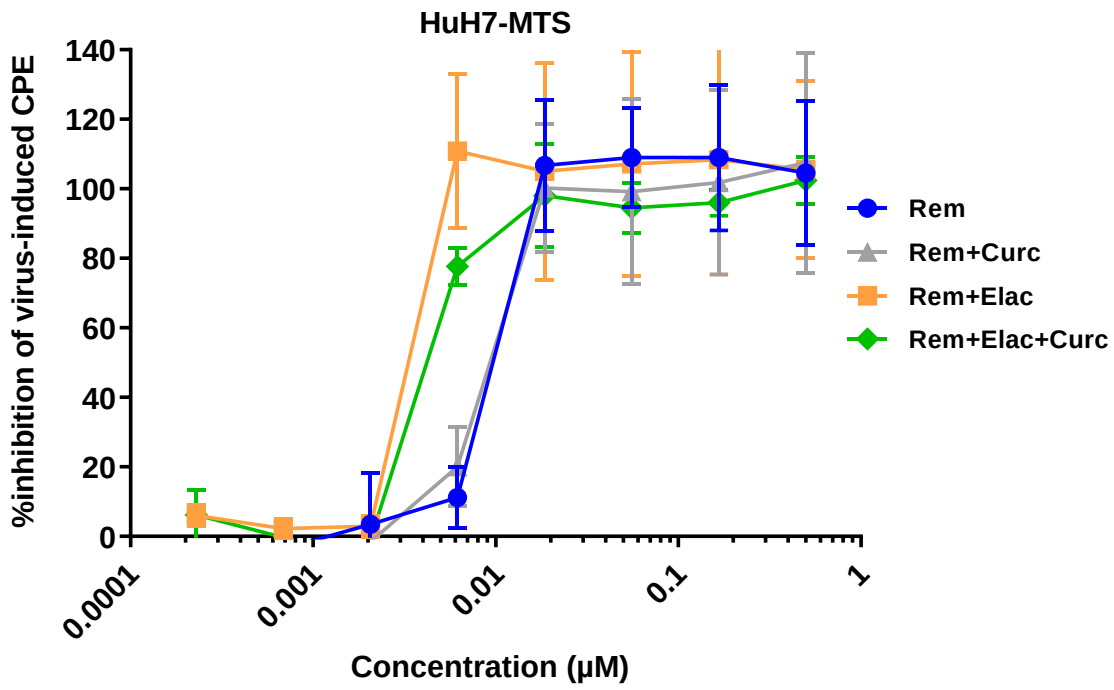
DOSE RESPONSE in HUMAN HuH7 cells (Remdesivir more potent at base)



Compound(s)	Concentration (µM)/HuH7 MTS		
	CC_{50}	EC_{50}	*X-fold
Rem	3 ±0.07	0.01 ±0.001	**1**
Rem+Curc	ND	0.009 ±0.002	**1.1**
Rem+Elac	ND	0.003 ±0.0005	**2.9**
Rem+Curc+Elac (REC)	ND	0.004 ±0.0002	**2.3**

EC_{50}, 50% effective concentration
CC_{50}, 50% cytotoxic/cytostatic concentration
**X-fold= EC_{50} remdesivir/EC_{50} combo*
ND= not determined
Data are mean values ± SD of at least three independent experiments.

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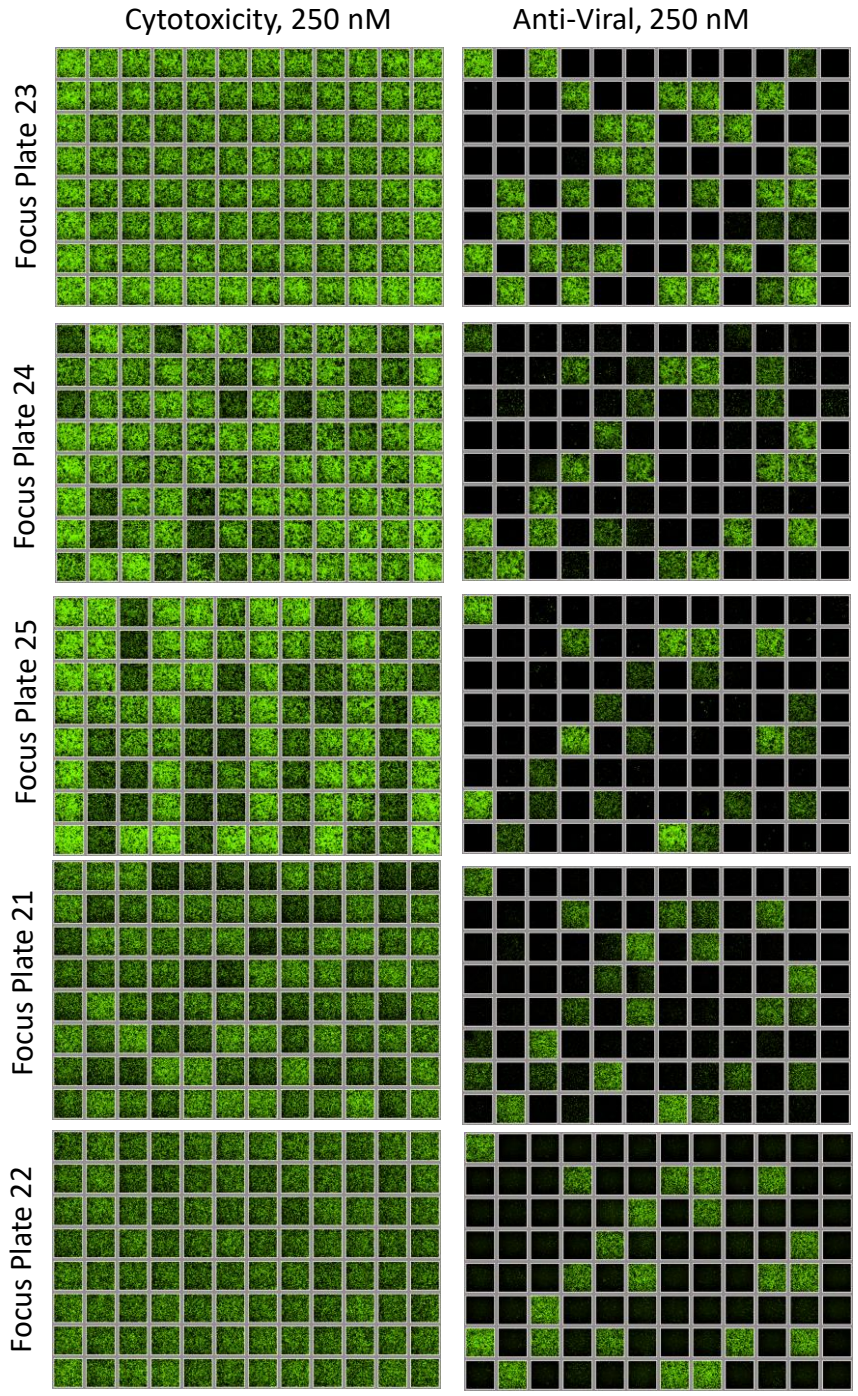
ESTABLISHING MOA



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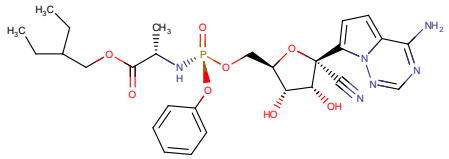
SUGGESTED COMBINATION MOA

- **REMDESIVIR**: ribonucleoside analogue, Chain Breaker during viral RNA production

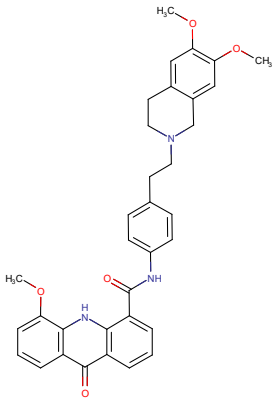
  Multiple functional ribonucleoside-type RNA polymerase inhibitors were tested to substitute REMDESIVIR in REC
  Galidesivir, Ribavirin, Favipiravir, the EIDD-class (molnupiravir) all failed
  The active metabolite of Remdesivir GS441524 also failed

- **ELACRIDAR/TARIQUIDAR**: ABCB1/ABCG2-dual inhibitors. Inhibiting cellular export of REMDESIVIR (both exporters are engaged)
- **CURCUMIN**: NRF2-mediated HO-1 activation is suggested MOA



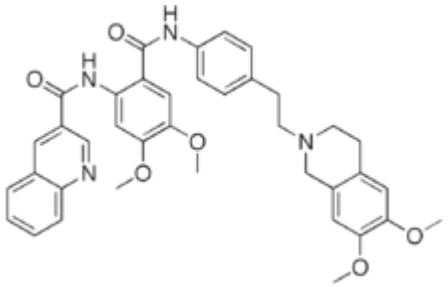
REMDESIVIR

+



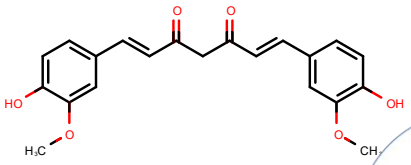
ELACRIDAR

or



TARIQUIDAR

+



CURCUMIN

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VALIDATION OF DUAL-SPECIFICITY ABCC-class inhibitors

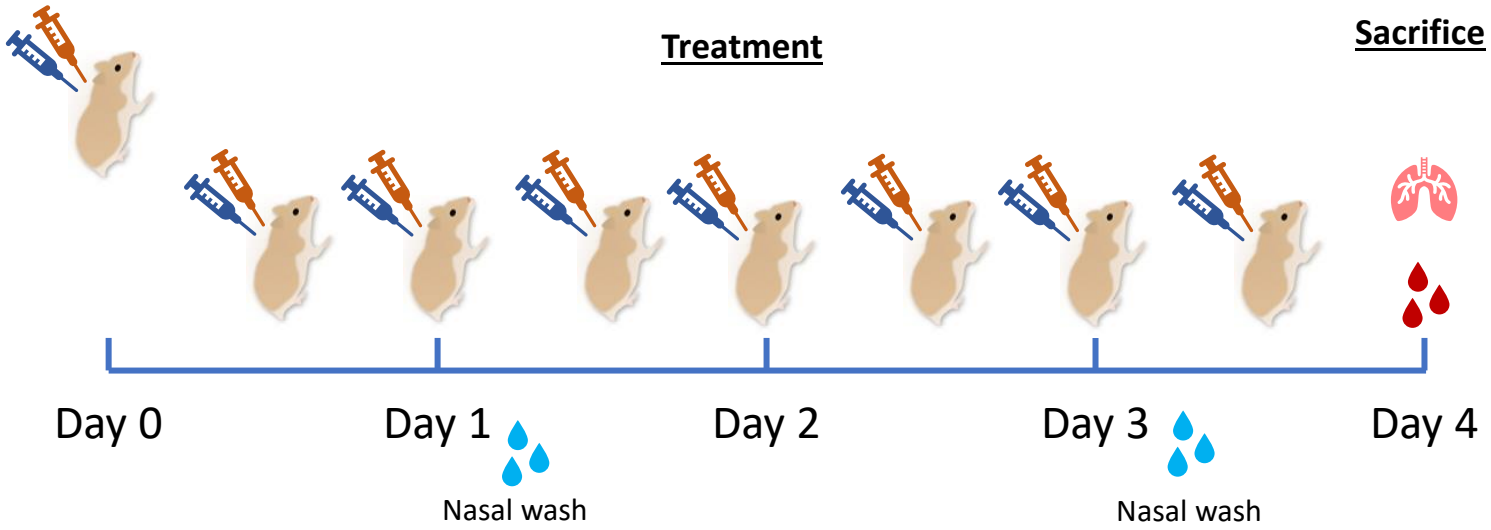
Compound(s)	Concentration (µM)/Vero E6 WT MTS		*X-fold
	CC_{50}	EC_{50}	
Remdesivir	**>10**	0.46 ± 0.2	**1**
Tariquidar	**>10**	>10	**/**
Zosuquidar	**>10**	1.9 ± 0.07	**/**
ONT-093	**>10**	3.4 ± 1.7	**/**
Elacridar	**>10**	1.07 ± 0.5	**/**
Rem+Tariq	**ND**	0.07 ± 0.003	**6.6**
Rem+Zosuq	**ND**	0.06 ± 0.03	**7.7**
Rem+ONT-093	**ND**	0.09± 0.02	**5.1**
Rem+Elacr	**>10**	0.05 ± 0.02	**9.2**

Study Design for Efficacy Trial at IITRI

Start Treatment

1) tariquidar IP pretreatment **(t = – 2 h)**
2) remdesivir SC (**2 h later, t= 0 min**)

Intranasal Infection
SARS-CoV-2
($5x10^4$ TCID50)
Immediately before dosing



Groups (N=10 per group)
Vehicle Control
50 mg/kg Remdesivir (SC)
25 mg/kg Remdesivir (SC) + 10 mg/kg Tariquidar (IP)
50 mg/kg Remdesivir (SC) + 10 mg/kg Tariquidar (IP)
25 mg/kg Remdesivir (SC) + 10 mg/kg Tariquidar (IP) + 25 mg/kg Curcumin (IP)
50 mg/kg Remdesivir (SC) + 10 mg/kg Tariquidar (IP) + 25 mg/kg Curcumin (IP)

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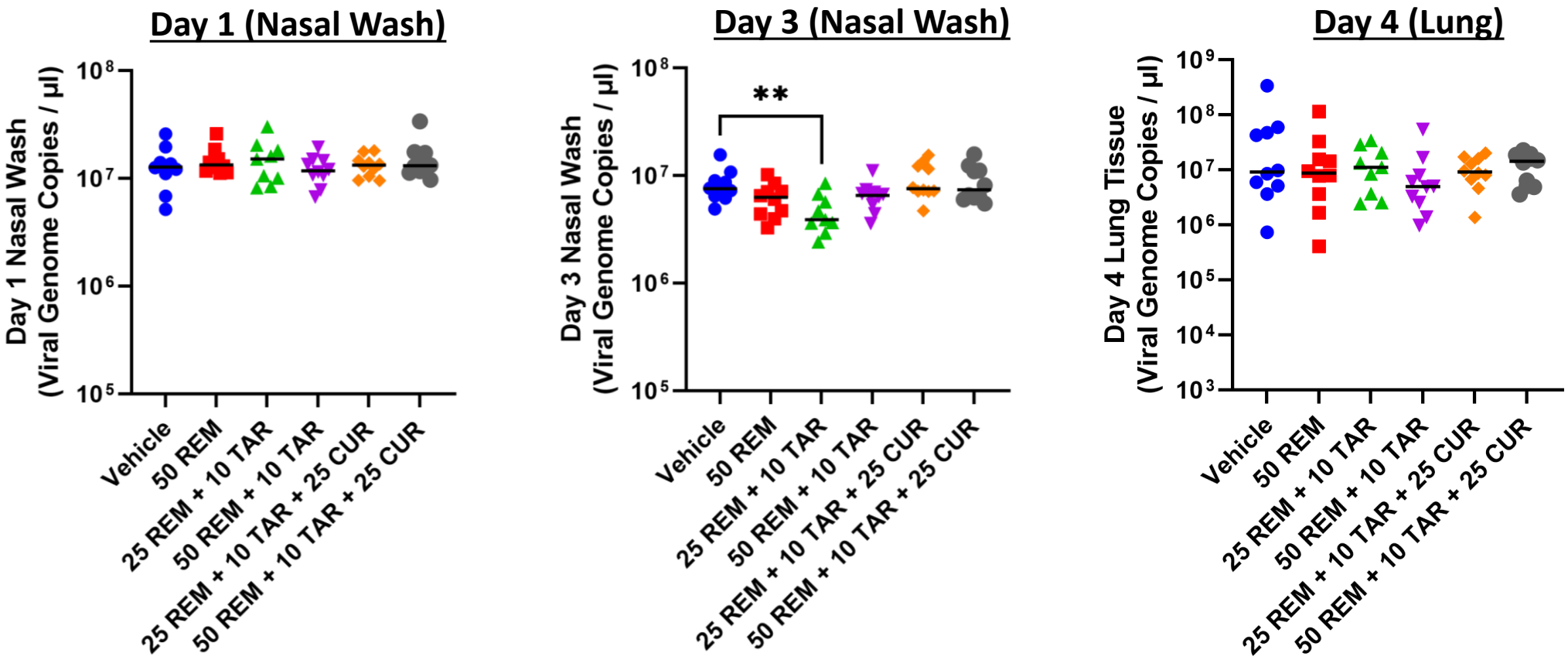
Minimal Antiviral Effect in Hamster Efficacy Trial



- Overall, treatments showed no/minimal benefit
  → Combination is ineffective *in vivo; OR* model is not appropriate and/or optimized

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Mann-Whitney Test: * indicates $p < 0.05$; ** indicates $p < 0.01$

Conclusions

- HD-DoE provides an effective method to survey combinations among candidate anti-viral drugs at reduced nM first-pass testing
- Results translate well into low nM combination identified
- Results consistent with a biologically justified MOA, which could quickly be validated using pathway selective alternatives
- The method appears broadly applicable to other viruses, and the screen can be made pre-fabricated to reduce response times
- In the case of the REC/RTC combination, however, we failed to demonstrate in-vivo efficacy against SARS-CoV2 viral load in lungs of Hamsters
  - Poor animal model?
  - Remdesivir metabolism in rodents a concern
  - Insufficient expression of ABCC-family genes in lung

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Thank you

ERINHA: Krista Versteeg and Audrey Richard

KUL: Pieter Leyssen, Johan Neyts, Rana Abdelnabi, Laura Vangeel