evotec

#RESEARCHNEVERSTOPS

Evotec

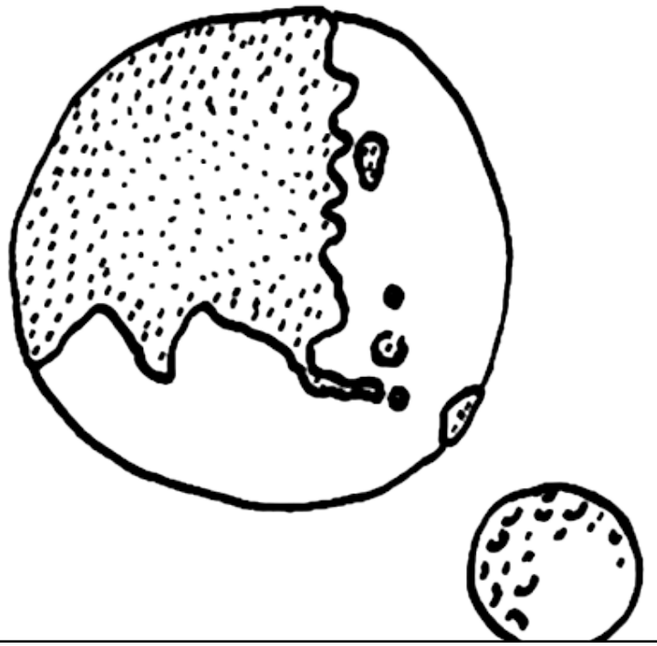
SARS-CoV-2 capabilities

Agenda

Evotec Overview

SARS-CoV-2 capabilities (*in vitro* and *in vivo*)

Summary



Relevant, seamless and state of the art

Integrated value chain

PDC

Best-in-class Drug Discovery & Early Development

- Translation from idea to development candidate
- Drug discovery solutions based on multidisciplinary capabilities across biology and chemistry

Preclinical studies & INDiGO

- Translation from *in-vitro* to patient
- Seamless transition from development candidate through to pre-clinic and IND

Small molecules high-end CMC

- GMP manufacturing for clinical development programmes and Niche scale commercial API and Drug Product

Biologics Molecule Optimization – J.MD™

- ML based sequence evaluation for lead selection of best molecules for rapid and efficient manu-facturability and formulation stability

Biologics process development – JP3®

- Formulation design from clinical to commercial with powerful analytical, ML and high-throughput tools

Biologics high-end CMC – J.POD®[1)]

- Modular and flexible clinical and commercial manufacturing at highest quality and low costs
- Seamless integration

[1)] J.POD is currently under construction for fastest possible initiation to build in USA and EU – expected 2021

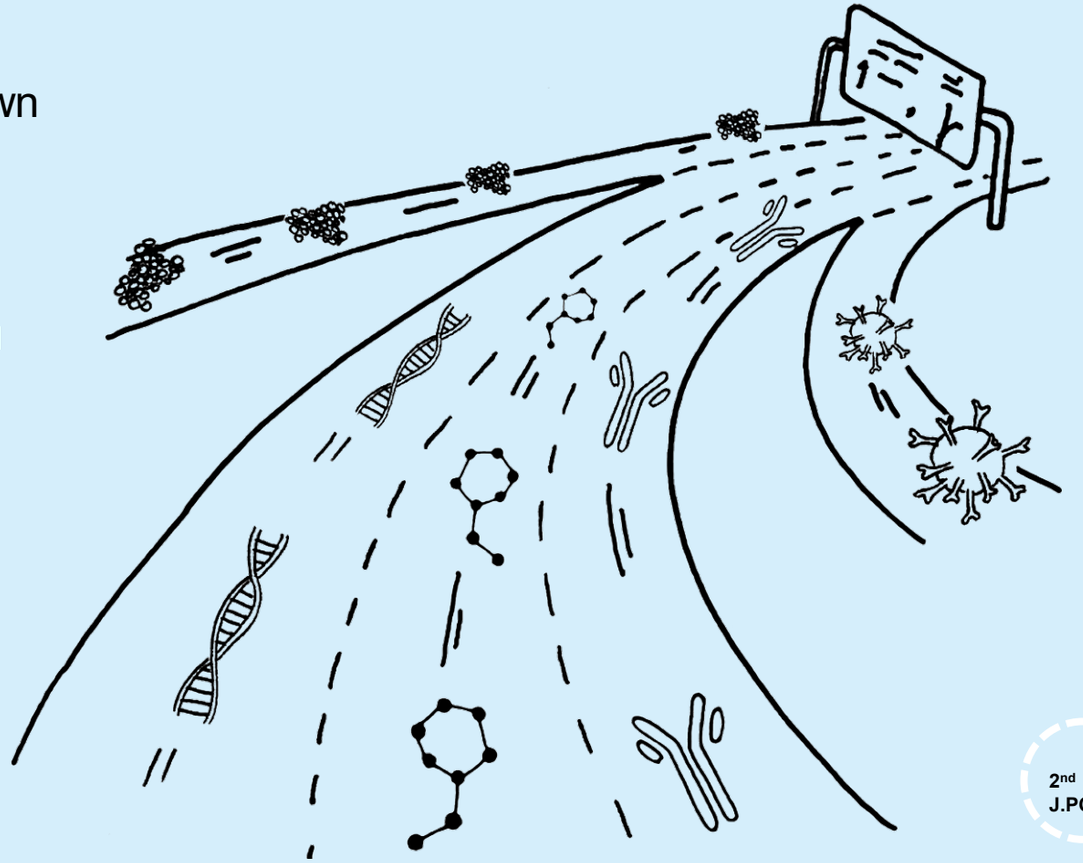
One platform – more efficiency, better precision, higher speed

Evotec footprint – 14 Sites & more than 4,000 employees

Princeton, Seattle, Branford, Watertown
~400 FTE

Orth an der Donau
~35 FTE

Verona (Campus Levi-Montalcini)
~700 FTE



Hamburg (HQ), Goettingen (Manfred Eigen Campus) Cologne, Munich,
~900 FTE

Abingdon (Dorothy Crowfoot Hodgkin), Alderley Park
~850 FTE

Lyon, Toulouse (Campus Curie)
~800 FTE

Evotec is confronting the renewed challenge in Infectious Diseases

Innovation and operational excellence

INNOVATION
Innovative thinking
Creativity
Novel approaches

INTEGRATION
Infrastructure
Knowledge

EFFICIENCY
Dedicated platforms
Expertise and
operational excellence

~200 Scientists dedicated to ID across multiple functions

Anti-Infective foundations – built upon years of experience

State-of-the art capabilities & extensive expertise

1 Experienced anti-infective drug discovery team with ~200 scientists covering natural products, HTS, medicinal chemistry, ADME, DMPK and disease specific biology

2 Experience encompasses multiple compound classes: Small molecules, natural products, biologics, peptides, antibodies, combinations, biocides, vaccines, antibacterials, antivirals, antifungals, antiparasitics

3 Multiple drug discovery approaches from phenotypic screening to target-based discovery: Folate, non-mevalonate, aromatic biosynthesis, protein synthesis, ribosome, virulence attributes & resistance pathways

4 **Extensive portfolio of drug discovery capabilities**

- Medicinal chemistry and structure-based drug design
- Computational approaches
- Hit finding & library screening
- Natural product libraries and deconvolution
- *In vitro* microbiology / virology / parasitology
- Molecular profiling / Mechanism of Action / Mechanism of resistance determination
- EvostrAIn™: strain collection
- *In vivo* models of infection, strongly coupled with PK/PD profiling expertise translating discovery data to clinical trial design
- Biophysical assays
- Immunobiology

Contribution to the discovery and development of multiple 'anti-infective' agents incl. preclinical and clinical candidates through to marketed drugs

Virology: a key portfolio at Evotec

Summary of capabilities

- Evotec has longstanding expertise in supporting virology programs
- Continuous development of viral disease biology capability and expertise
- Investment in bolstering the platform for respiratory viruses (including coronaviruses) as well as HBV
- *In vitro* capabilities include
  - Culture of virus in suitable cell lines
  - Culture of HBV in primary hepatocytes
  - Antiviral assay by CPE with additional antiviral assays endpoints including ToxGlo and qPCR
  - ELISA against virus specific proteins
  - Neutralisation assay
  - Selection of resistant virus
- Infection and survival models in suitable animal hosts
- Additional endpoints include viral load (culture/qPCR etc), biomarkers, cytokines, antibody response
- Pathogen associated and host response

Evotec Viral Screening Capabilities

Ability to screen at BSL2/BSL2^{+} & BSL3

- >15 years of screening expertise in anti-infective space
  - Antibacterials (e.g. ESKAPE, Gram positive, *Mycobacterium*, …)
  - Antivirals (e.g. HBV, hPIV, RSV, HIV, rhinovirus, hMPV …)
- Medium (96- & 384-well format) to High Throughput (1536-well format) Screening with RTqPCR, phenotypic or target-based readouts
- Support for back screening and hit expansion and secondary assays for hit characterization
- BSL2/BSL2^{+} screening capabilities for HTS/MTS
  - 2 BSL2/BSL2^{+} HTS platforms (ET-1/2) and 1 BSL2 MTS platform (Agilent workstation)
- BSL3 screening capabilities for MTS
  - BSL3 lab (~200 m^{2}) including controlled access, autoclave and H_2O_2 SAS for liquid & solid waste handling
  - BSL3 trained people (HBV, Tuberculosis)
  - Basic equipment [safety cabinets (3), incubators, refrigerators and freezers (-20°C and -80°C)]
  - Automation equipment under safety cabinet (2)
    - Dispenser (Multidrop + S-Lab) for cell/reagent dispensing and Plate Sealer
    - Pipetor (Cybiwell, Cybio) for compound addition
    - Multimode plate reader (EnVision) with stackers (30 plates)
  - Screening in semi-automatic process (384-well format)

ET-1

ET-2

BSL3

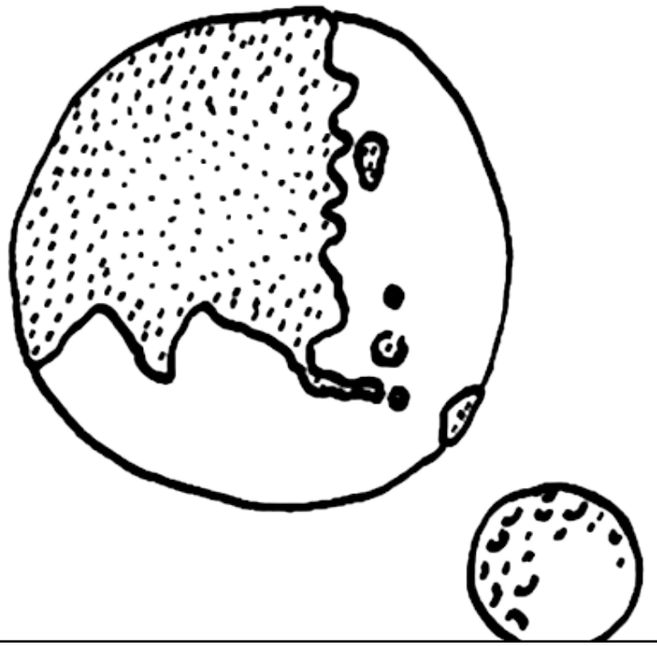
BSL3

Agenda

Evotec Overview

SARS-CoV-2 capabilities (*in vitro* and *in vivo*)

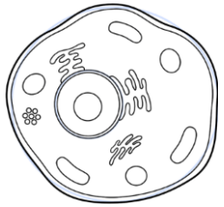
Summary



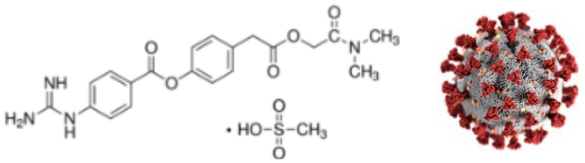
SARS-CoV-2 infection model

CellTiter Glo assay: restricted to cell lines with CPE (VeroE6)

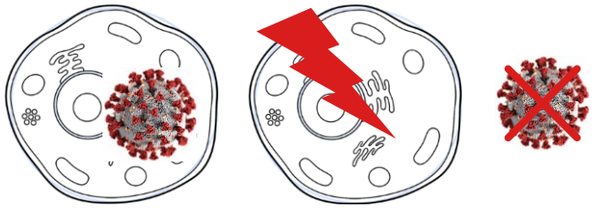
Seed Cells



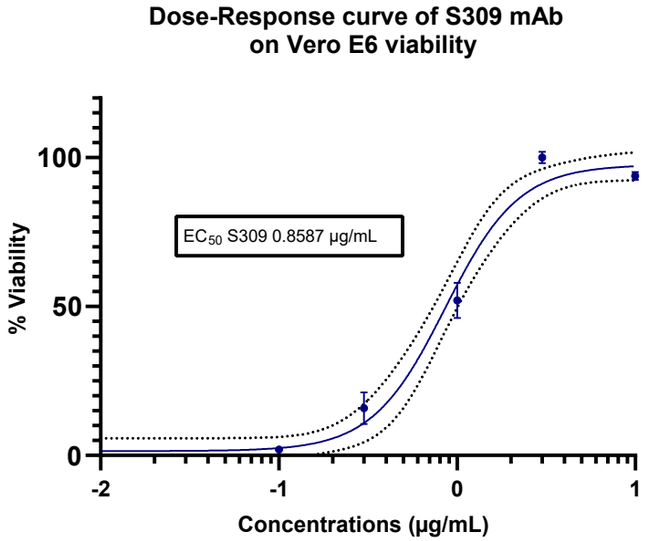
Add Compound + Virus

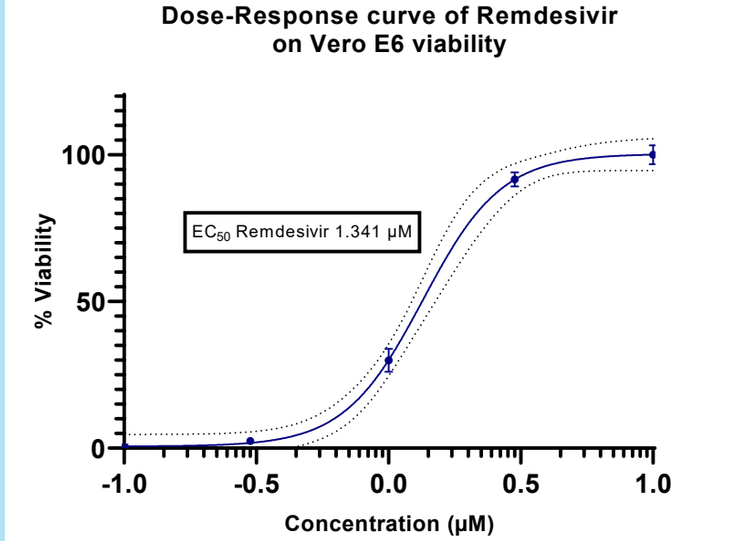


Measure Cell Viability



SARS-CoV-2 infection



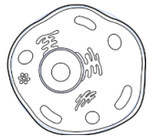


S309 mAb and Remdesivir dose response curves in Vero E6 cells infected with SARS-CoV-2, CellTiter Glo™ 3 dpi

SARS-CoV-2 infection model

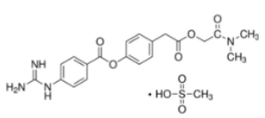
RT-qPCR analysis

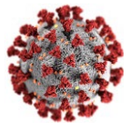
Seed Cells



- **A549-hACE2**
- **A549-hACE2/hTMPRSS2**
- **Calu-3**

Add Com-pound + Virus





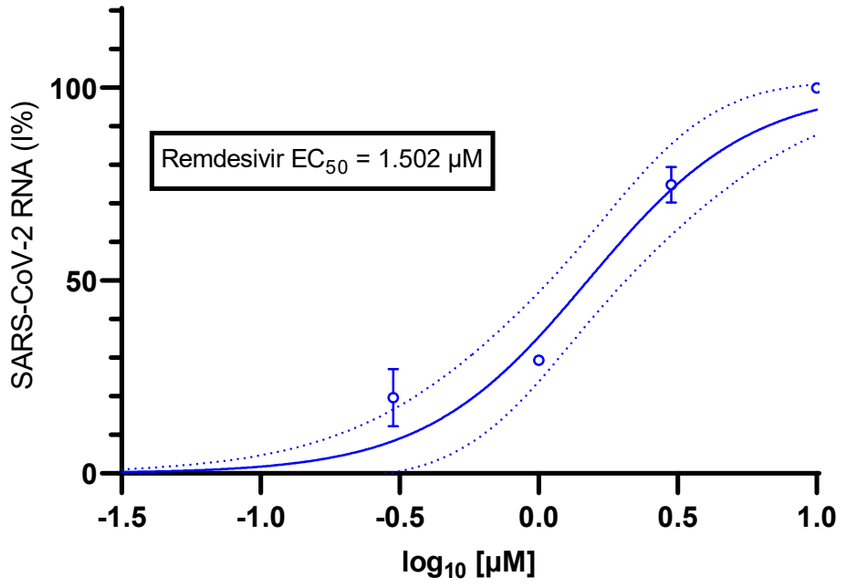
3d incubation

Cell lysis and RNA extraction

RT-qPCR

- **Cell lysates**
- **Supernatants**

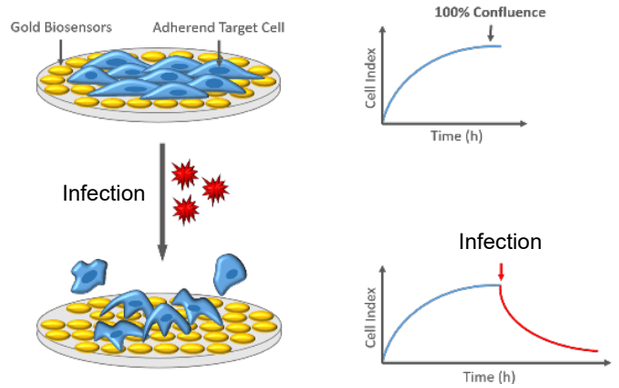
Effect of Remdesivir on SARS-CoV-2 RNA quantification by RT-qPCR in cell lysates



SARS-CoV-2 infection model

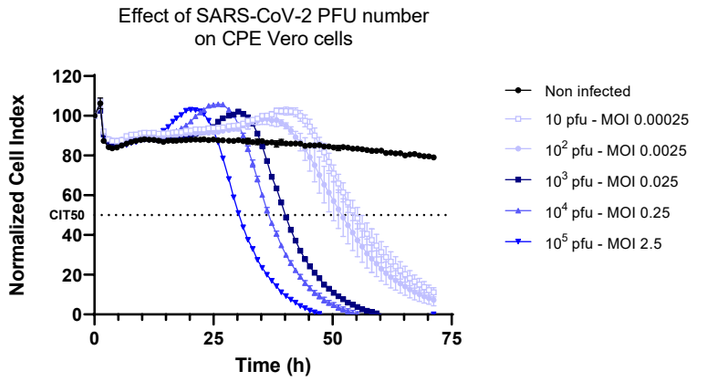
xCELLigence – Vero E6

xCELLigence principle



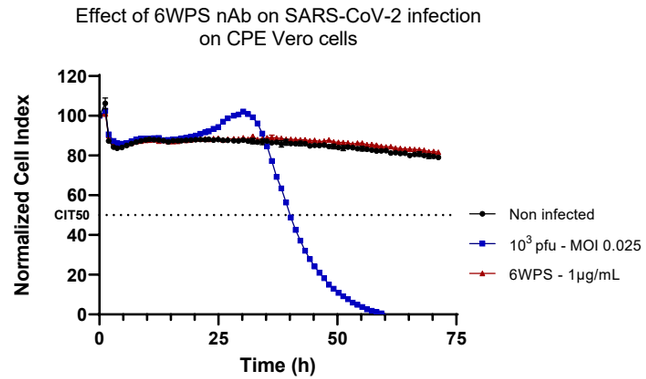
- Vero cells in 96w E-plate – 4 x10^4 cells/well
- Strain SARS-CoV-2 USA-WA1/2020
- Tested number of PFU: from 10 to 10^6
- 72h infection
- Detection of electrical impedance every 15 min

Effect of PFU



Good correlation observed between cell impedance and the number of PFU

Effect of 6WPS mAb



6 WPS neutralizing mAb protects from CPE triggered by SARS-CoV-2

Currently 9 variants available including:

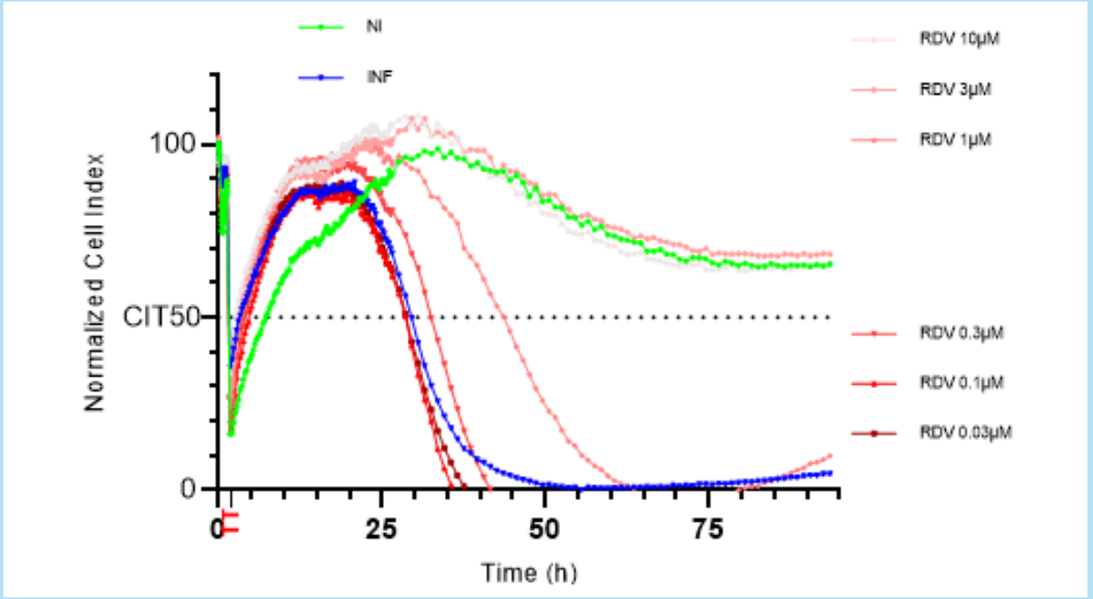
- Delta variant
- Brazil/Japan, England, South Africa
- Denmark
- USA

PFU 2-fold difference in CIT_{50}

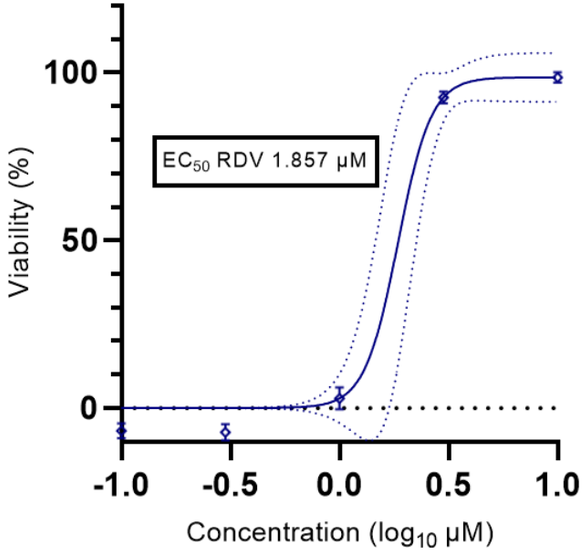
SARS-CoV-2 infection model

xCELLigence – Vero E6

Effect of Remdesivir



Determination of EC_{50}

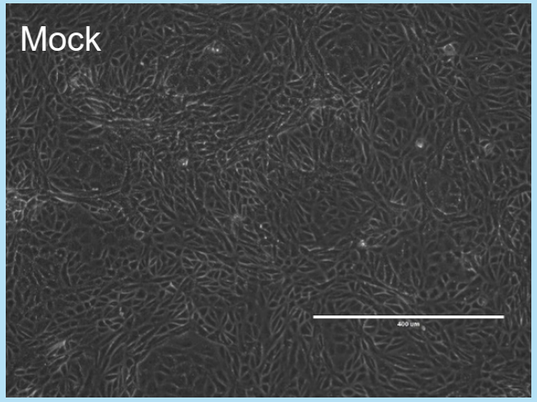


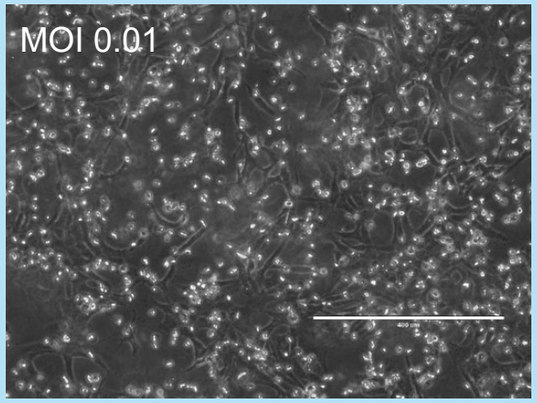
Remdesivir inhibits SARS-CoV-2 infection in a dose dependent manner, that allows determination of EC_{50}

Plaque-forming Assay

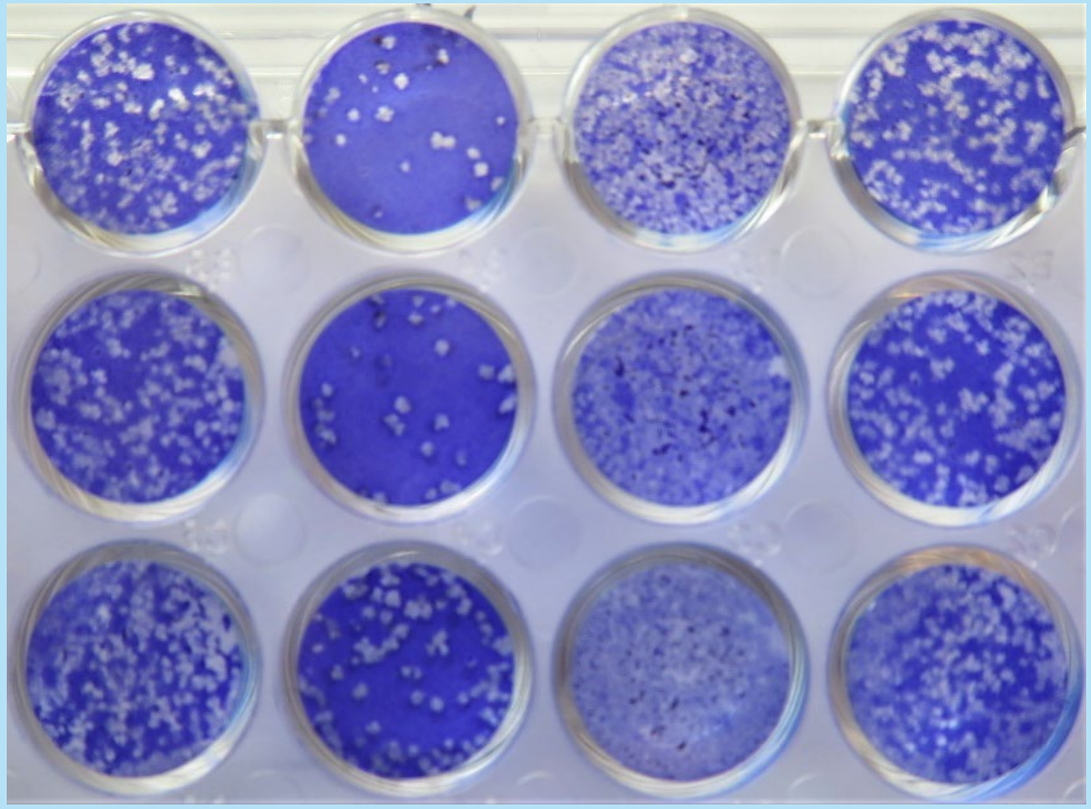
Titration of SARS-CoV-2

- SARS-CoV-2 viral culture in Vero E6 cells
  - Suitable for low to medium throughput assays,
- SARS-CoV-2 plaque assay
  - Quantification and validation of viral stocks
  - Determination of viral burden in tissue, e.g. as read-out for *in vivo* studies
  - Generation of resistant virus
  - Mechanistic studies





Virus-induced CPE



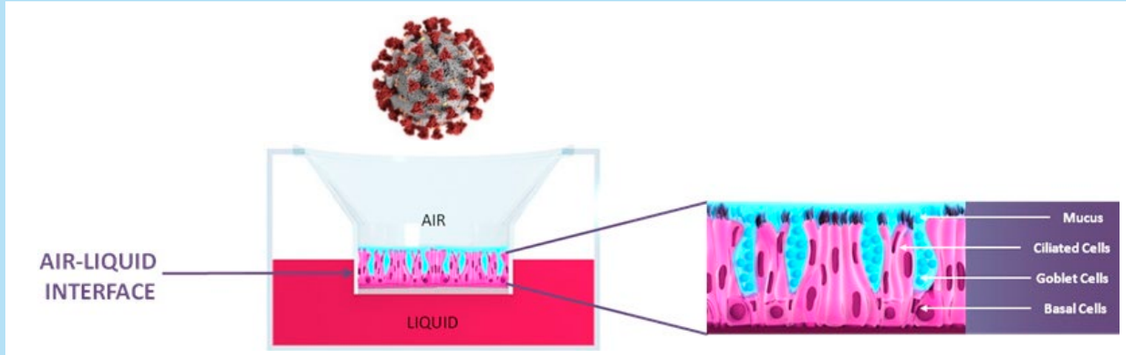
Several SARS-CoV-2 variants are available

Virus (original stock)	Lineage	GISAID clade	Comments
SARS-CoV-2 Germany/BavPat1/2020	B	G	–
SARS-CoV-2 USA-WA1/2020	A	S	–
SARS-CoV-2 Chile/Santiago_op4d1/2020	A.2	S	–
SARS-CoV-2 England/02/2020	A	S	–
SARS-CoV-2 hCoV-19/England/204820464/2020	B.1.1.7	GR	Alpha
SARS-CoV-2 hCoV-19/South Africa/KRISP-EC-K005321/2020	B.1.351	GH	Beta
SARS-CoV-2 hCoV-19/South Africa/KRISP-K005325/2020	B.1.351	GH	Beta
SARS-CoV-2 Hong Kong/VM20001061/2020	A	S	–
SARS-CoV-2 Italy-INMI1	–	O	–
SARS-CoV-2 New York 1-PV08001/2020	B.4	O	–
SARS-CoV-2 New York-PV08410/2020	B.1	GH	–
SARS-CoV-2 New York-PV08449/2020	B.1	GH	–
SARS-CoV-2 New York-PV09158/2021	B.1.3	GH	–
SARS-CoV-2 New York-PV09197/2020	B.1.3	GH	–
SARS-CoV-2 Singapore/2/2020	B	L	–
SARS-CoV-2 USA/CA_CDC_5574/2020	B.1.1.7	GR	Alpha
SARS-CoV-2 USA-AZ1/2020	A	S	–
SARS-CoV-2 USA-CA1/2020	A	S	–
SARS-CoV-2 USA-CA2/2020	B.2	O	–
SARS-CoV-2 USA-CA3/2020	B	L	–
SARS-CoV-2 USA-CA4/2021	B	L	–

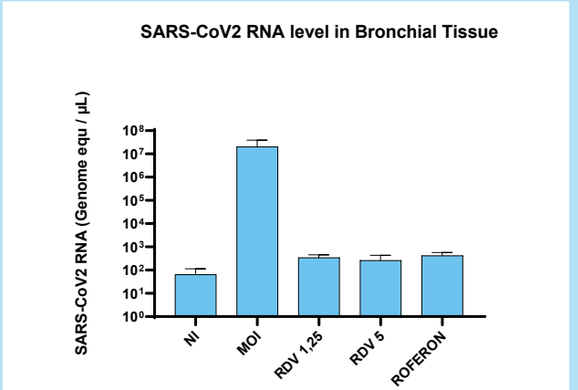
Virus (original stock)	Lineage	GISAID clade	Comments
SARS-CoV-2 USA-IL1/2020	B	O	–
SARS-CoV-2 USA-WI1/2021	B	L	–
SARS-CoV-2 Canada/ON/VIDO-01/2020	B	L	–
SARS-CoV-2 hCoV-19/Scotland/CVR837/2020	B.1.5	G	–
SARS-CoV-2 hCoV-19/Scotland/CVR2224/2020	B.1.222	G	–
SARS-CoV-2 hCoV-19/Denmark/DCGC-3024/2020	B.1.1.298	GR	–
SARS-CoV-2 hCoV-19/Japan/TY7-503/2021	P.1	GR	Gamma
SARS-CoV-2 hCoV-19/USA/CA-Stanford-15_S02/2021	B.1.617.1	G	Kappa
SARS-CoV-2 hCoV-19/USA/NY-NP-DOH1/2021	B.1.526	GH	Iota
SARS-CoV-2 hCoV-19/USA/MD-HP01542/2021	B.1.351	GH	Beta
SARS-CoV-2 hCoV-19/USA/PHC658/2021	B.1.617.2	GH	Delta
SARS-CoV-2 hCoV-19/Peru/un-CDC-2-4069945/2021	C.37	GR	Lambda
SARS-CoV-2 hCoV-19/USA-NJ-CVD124/2020	R.1	GR	–
SARS-CoV-2 hCoV-19/USA/OR-OHSU-PHL00037/2021	B.1.1.7	GRY	Alpha
SARS-CoV-2 USA/CA/VRLC012/2021	P.2	GR	Zeta
SARS-CoV-2 USA/CA/VRLC014/2021	B.1.429	GH	Epsilon
SARS-CoV-2 USA/CA/VRLC009/2021	B.1.427	GH	Epsilon
SARS-CoV-2 hCoV-19/USA/MD-HP05285/2021	AY.24	GK	Delta
SARS-CoV-2 hCoV-19_USA_MD-HP05647_2021	B.1.617.2	GK	Delta
SARS-CoV-2 hCoV-19/USA/CA-VRLC086/2021	AY.1	GK	Delta
SARS-CoV-2 hCoV-19/USA/MD-HP03056/2021	B.1.525	G	Eta
SARS-CoV 2. hCoV-19/USA/MD-HP20874/2021	B1.1.529	GR	Omicron

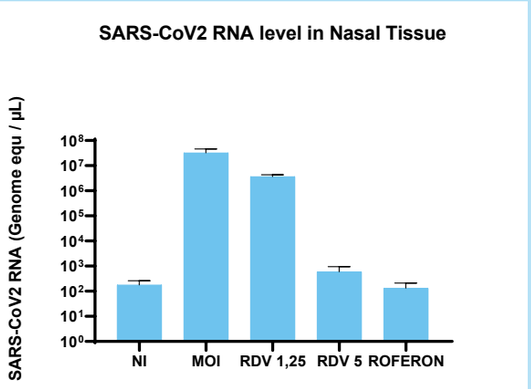
Human airway epithelial assay

Initial data on SARS-Cov-2



Schematic representation of MucilAir™ (adapted from http://www.epithelix.com/products/mucilair)

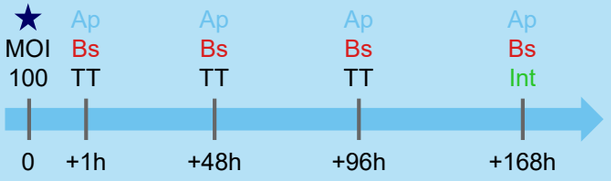




- MucilAir™ is a human *in vitro* model, representing the upper airway epithelia containing beating cilia, goblet and basal cells
- The model is a highly relevant and useful tool to address pharmacology, toxicology and biology demands
- Endpoint is viral load quantified by RT-PCR on basal, apical and intracellular compartments
- Other readouts available: viral load, host related parameters

Experimental set-up in 24 well plate!

Treatment and timings are adjustable



★ Viral inoculation

TT Treatment with test compounds

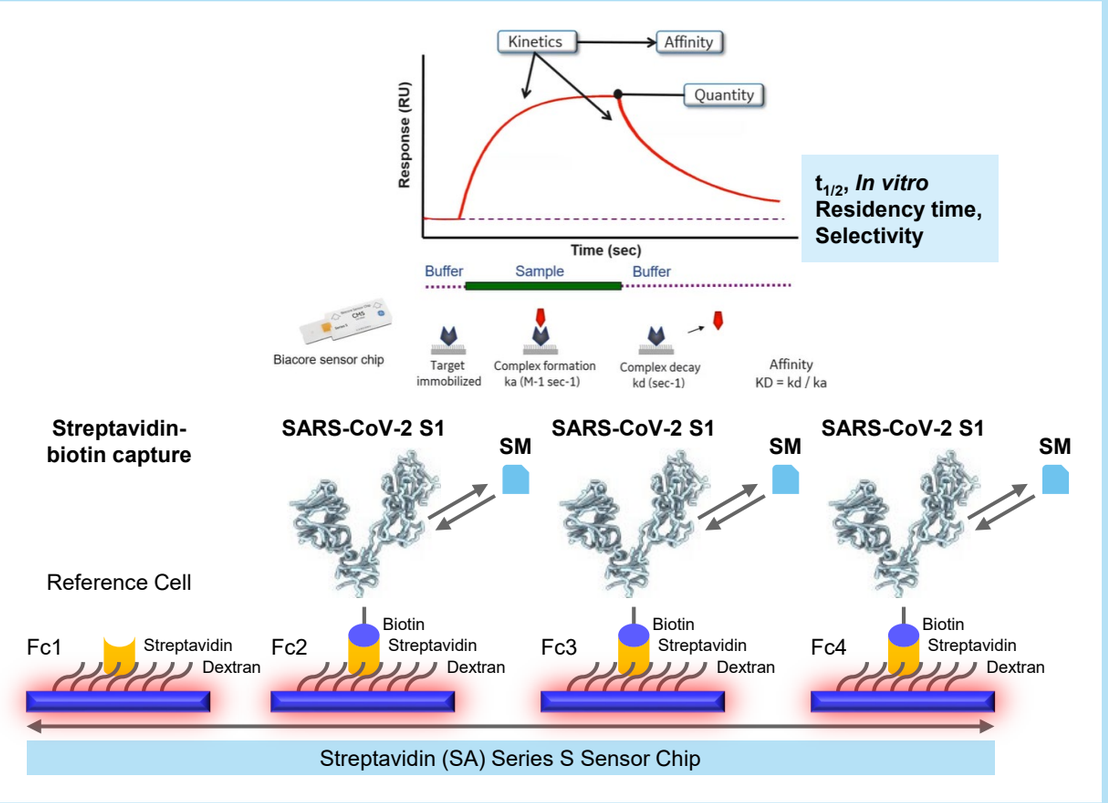
Ap Apical Washes (viral collection & RNA quantification)

Bs Basal collection for viral and Cytokine analysis

Int Intracellular viral quantification

SARS-CoV-2 *in vitro* pharmacology assays

- Background
  - Coronavirus uses spike glycoproteins found on the envelope to gain entry into the cells
  - Spike proteins are homotrimers comprising S1 and S2 subunits
  - SARS-CoV-2-Receptor Binding Domain (RBD) binds ACE2 receptor with high affinity, triggering S2 to divide from S1 and transit to a more stable post-fusion state, that is essential for membrane fusion
- Identification of molecules able to bind Spike subunit S1
  - SPR-based Assay with Biotinylated SARS-CoV-2 S1 protein, His, Avitag™
- Identification of molecules able to disrupt SARS-CoV-2-RBD/ ACE2 binding
  - Alpha Assay technology

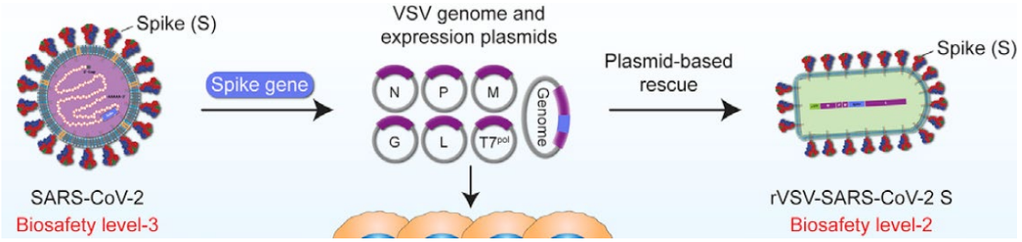


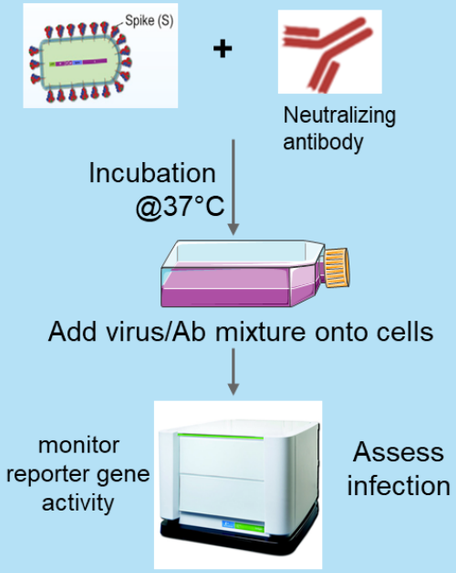
SARS-CoV-2-Spike pseudotyped infection

Pseudotyped viruses with different variants are available

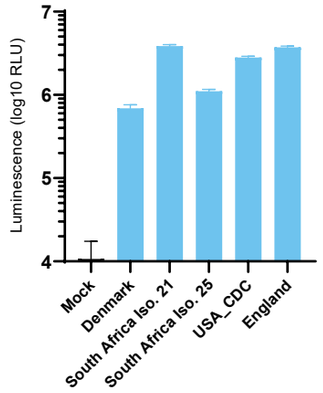
Generation of VSV bearing SARS-CoV-2 spike gene (rVSV-SARS-CoV-2)

- Spike from 9 different variants available
- 3 additional variants under construction

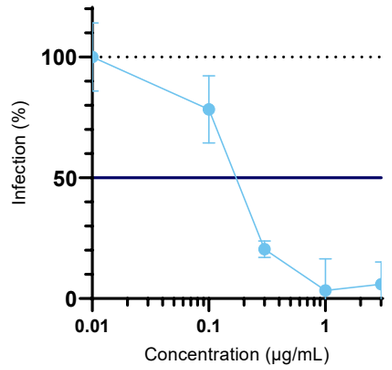




Generation of VSV bearing SARS-CoV-2 spike variants



Neutralization assay of rVSV-SARS-CoV-2 Spike



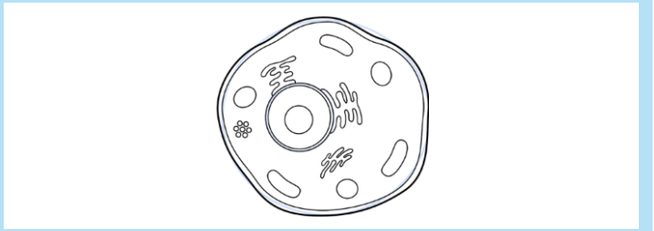
Available Spike variants for pseudotyping

Isolate	Lineage	GISAID clade	Comments
SARS-CoV-2 USA-WA1/2020	A	S	–
SARS-CoV-2 USA-WA1/2020, D614G	A	S	–
SARS-CoV-2 hCoV-19/England/204820464/2020	B.1.1.7	GR	Alpha
SARS-CoV-2 hCoV-19/South Africa/KRISP-EC-K005321/2020	B.1.351	GH	Beta
SARS-CoV-2 hCoV-19/South Africa/KRISP-K005325/2020	B.1.351	GH	Beta
SARS-CoV-2 USA/CA_CDC_5574/2020	B.1.1.7	GR	Alpha
SARS-CoV-2 hCoV-19/Denmark/DCGC-3024/2020	B.1.1.298	GR	–
SARS-CoV-2 hCoV-19/Japan/TY7-503/2021	P.1	GR	Gamma
SARS-CoV-2 hCoV-19/USA/CA-Stanford-15_S02/2021	B.1.617.1	G	Kappa
SARS-CoV-2 hCoV-19/USA/PHC658/2021	B.1.617.2	GH	Delta
SARS-CoV-2 hCoV-19/Peru/un-CDC-2-4069945/2021	C.37	GR	Lambda
SARS-CoV-2 hCoV-19/Columbia	B.1.621.1	GH	Mu
SARS-CoV 2. hCoV-19/USA	B.1.1.529	GR	Omicron

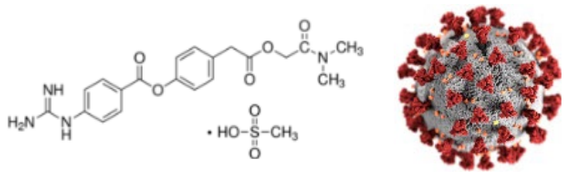
Other endemic human coronavirus strains

Screening and profiling assays under BSL2 containment

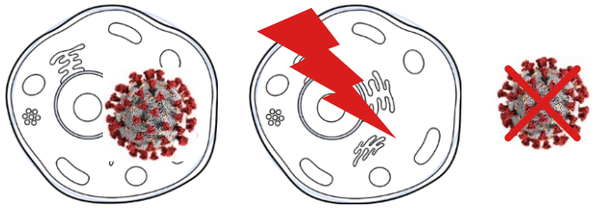
Seed Cells



Add Compound + Virus

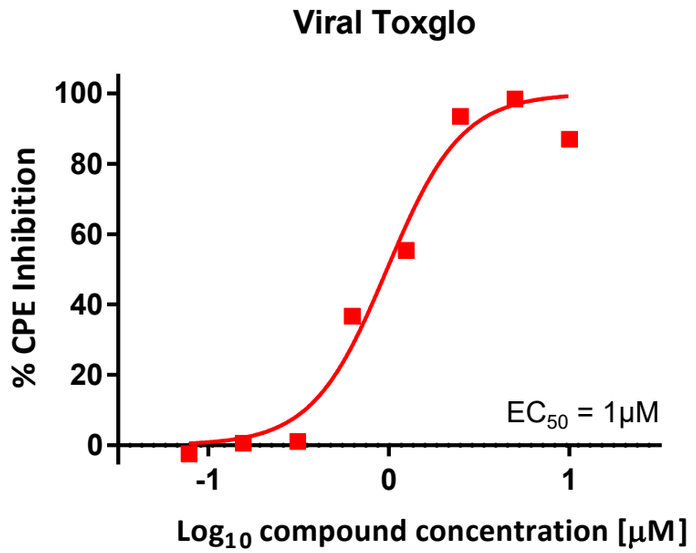


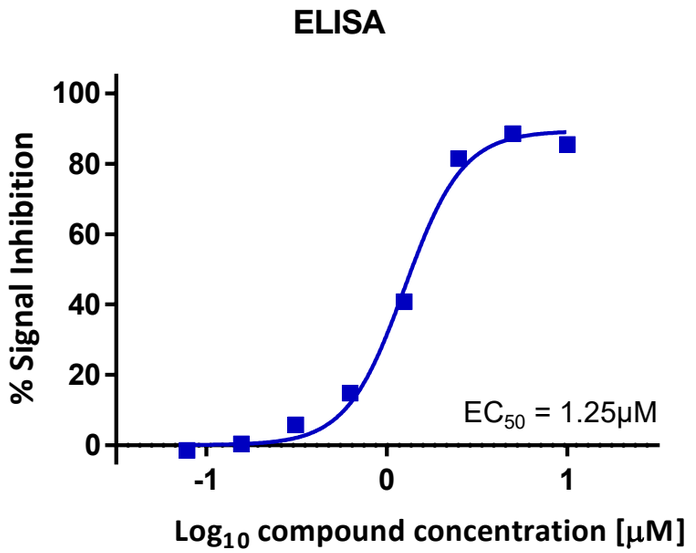
Measure Cell Viability

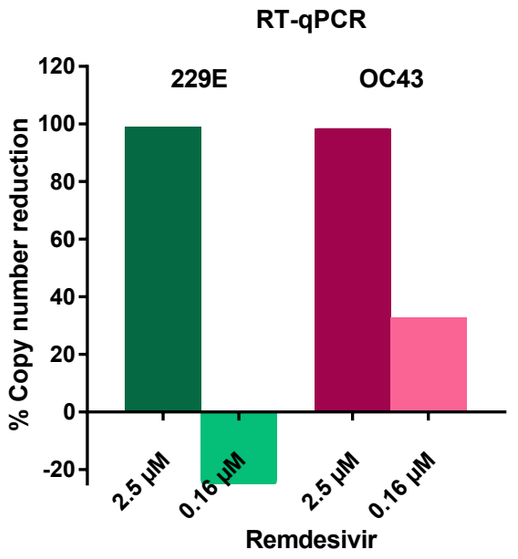


- Routine cellular screening assays against endemic human coronavirus strains
  - 229E, NL63 and OC43 (alpha and beta coronavirus)
- Standard assay set up:
  - Cells seeded
  - Infected with virus, treated with compound
  - Incubation for 72h (229E) or 144h (OC43)
  - Cell viability measured by Viral Toxglo™
- Assay can be performed in 96-well or 384-well format
- All plates containing uninfected cell control, virus control and inhibitor control (remdesivir)
- Cell lines with different properties can be selected depending on intended target
  - Limited number of cell lines where virus causes cytopathic effect (MRC-5, HeLa) for Viral Toxglo™ assay
  - ELISA against OC43 can be used also in cell lines where virus does not cause CPE (CaCo2, A549)
- Cytotoxicity testing in the respective infected cell line performed in parallel to identify potential false negatives
  - Standard cytotoxicity cell lines, e.g. THP-1 or HepG2 can be added to the testing cascade

Different endpoints for human coronavirus assays







Remdesivir dose response curves.
MRC-5 cells infected with OC43, Viral Toxglo 6dpi, ELISA 5dpi.

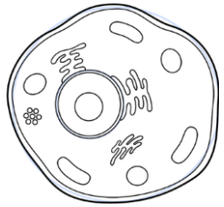
MRC-5 cells infected with 229E or OC43,
RT-qPCR 3dpi

Different endpoints are available to complement / confirm findings

- Viral Toxglo™ restricted to cell lines with CPE
- ELISA and RT-qPCR can be used for all cell lines but with lower throughput

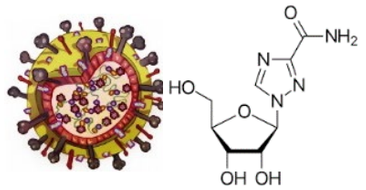
Live virus compound testing for respiratory viruses

Seed Cells



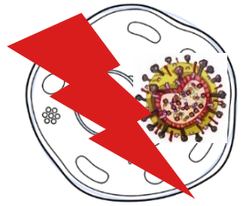
24h

Add Compound + Virus



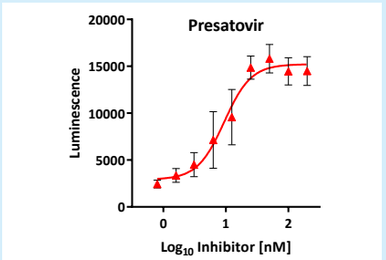
72h

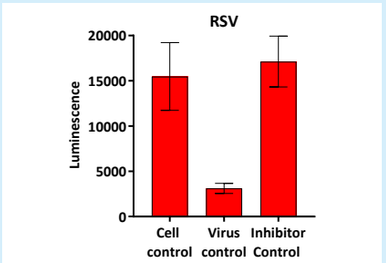
Measure Cell Viability/Virus enzyme



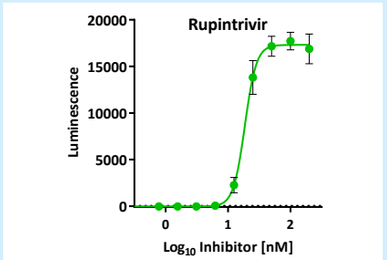
- Under BSL2 conditions
- 96-well or 384-well format, up to 1% DMSO tolerated
- All plates containing uninfected cell control, virus control and inhibitor control
- Robust assay window and z'-values

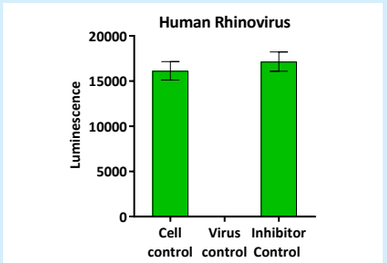
RSV



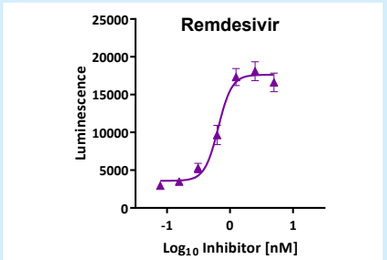


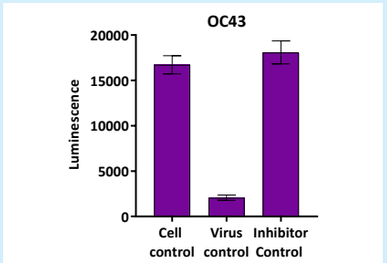
HRV



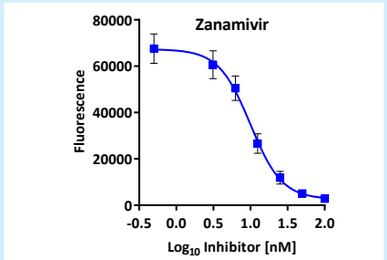


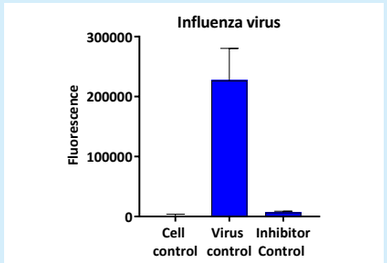
Human coronavirus OC43





Influenza A virus

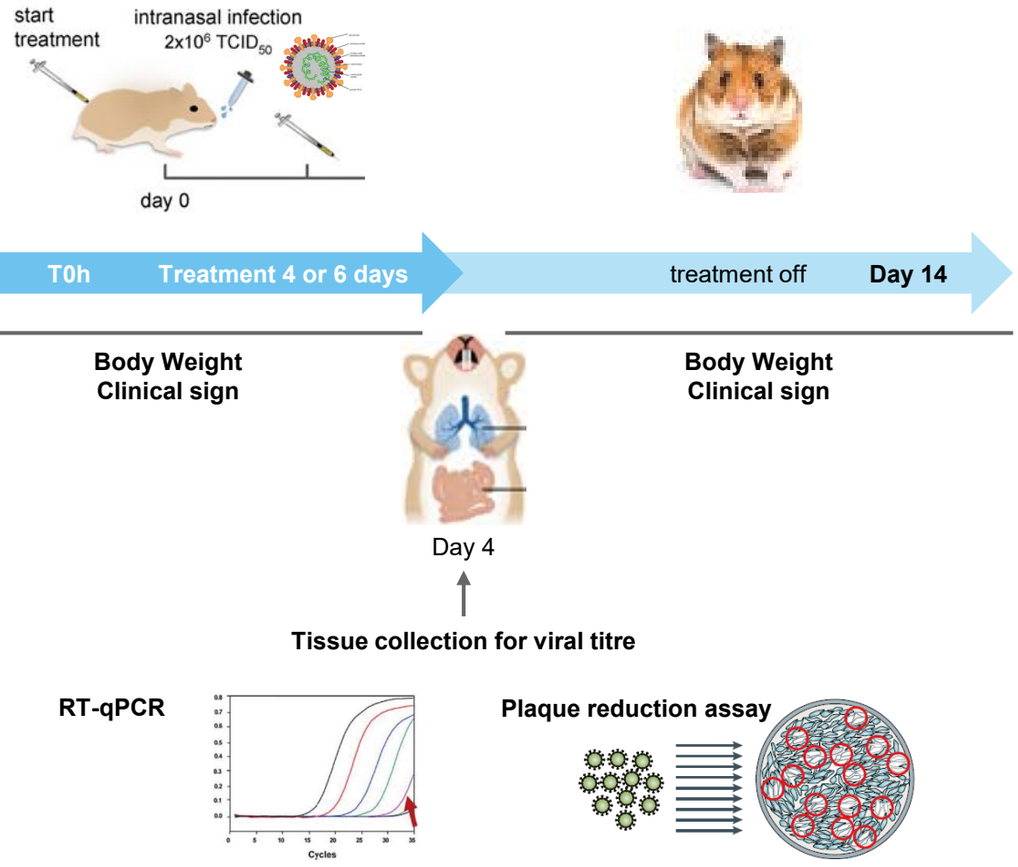




SARS-CoV-2 Hamster Model

Study design

- Golden Syrian Hamster
- Infection intranasally
- Several strains in development
- Dose range
- Monotherapy or combination
- Treatment with small molecule, Biology, Vaccine
- Bespoke dosing regimen
- Bespoke treatment duration & Study duration



Standard endpoints

- Virus titre in tissues and throat swabs
  - RT-qPCR
  - Plaque assay
- Clinical observations
  - Body weight
  - Health Scoring

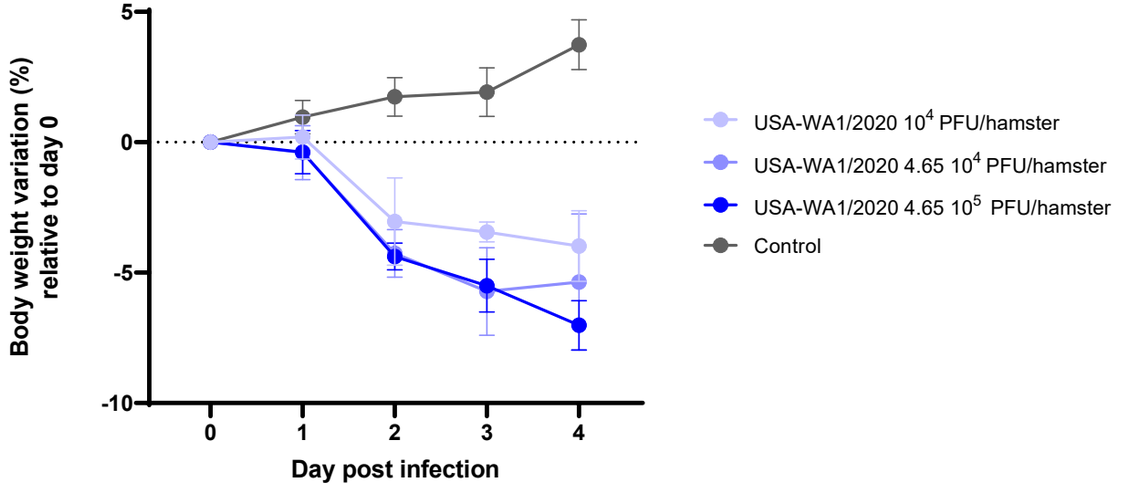
Additional endpoints

- Histopathology
- Immune response
  - Cytokines
  - Serology
- PK analysis

SARS-CoV-2 Hamster model development

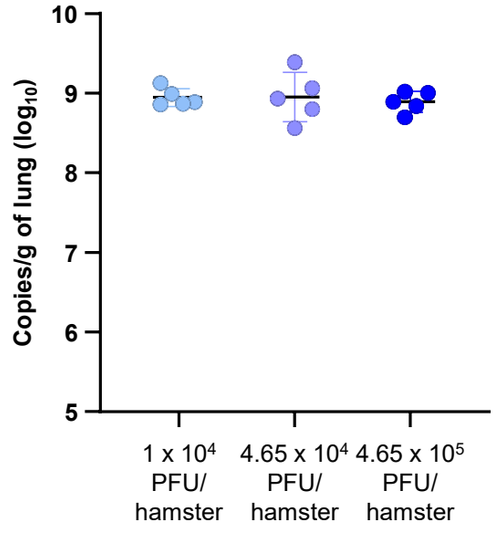
Examples of Study Endpoints

Body weight

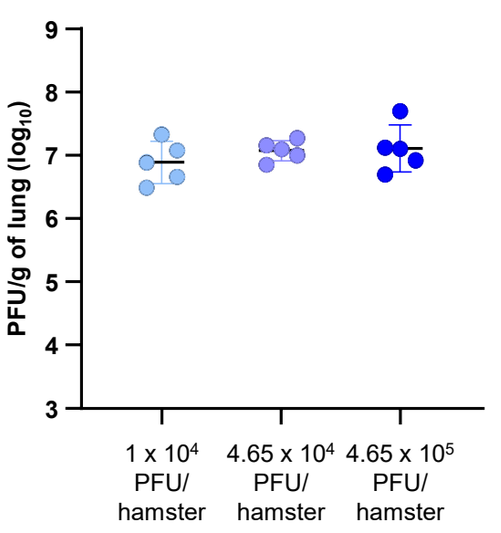


Virus strain USA-WA1/2020,
Body weight loss induced by SARS-CoV-2 Infection

RT-qPCR



Plaque assay



Virus titre on day 4 determined in lung tissue by RT-qPCR and plaque assay

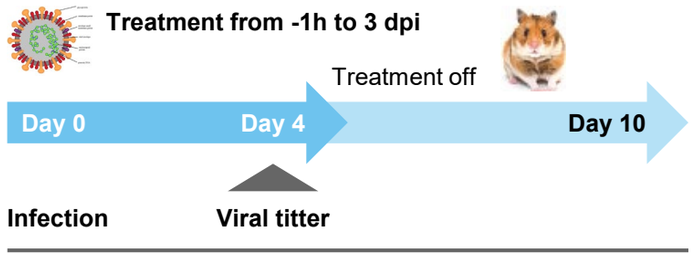
Average ± SD

SARS-CoV-2 Hamster model

Validation with reference compound

Infection

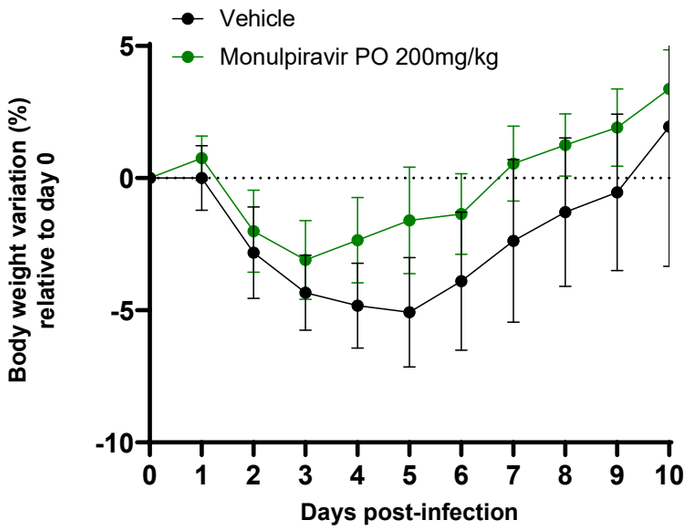
- SARS-CoV-2 USA-WA1/2020



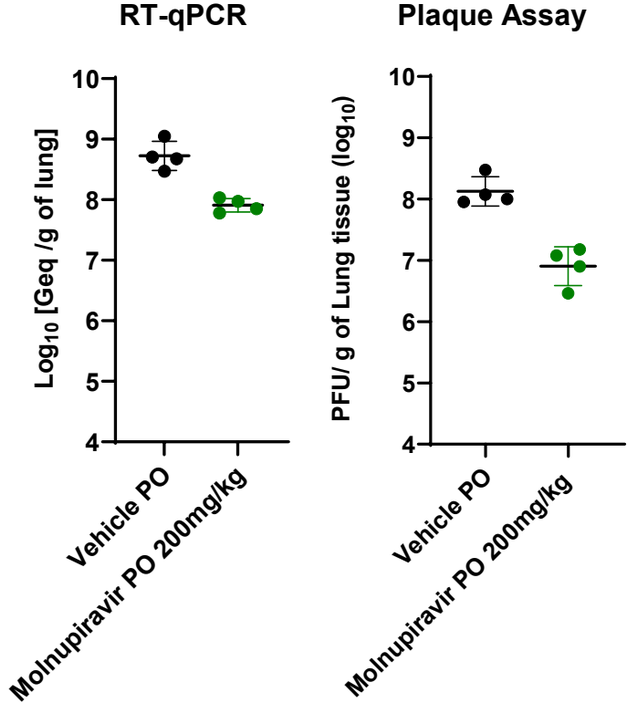
Body Weight / Clinical sign

Treatment	Treatment route and regimen	Tissue collection (day post infection)	End of experiment (day post infection)	No. Hamster
Vehicle	PO BID	4	4	4
		–	10	8
Molnupiravir 200mg/kg	PO BID	4	4	4
		–	10	8

Body weight

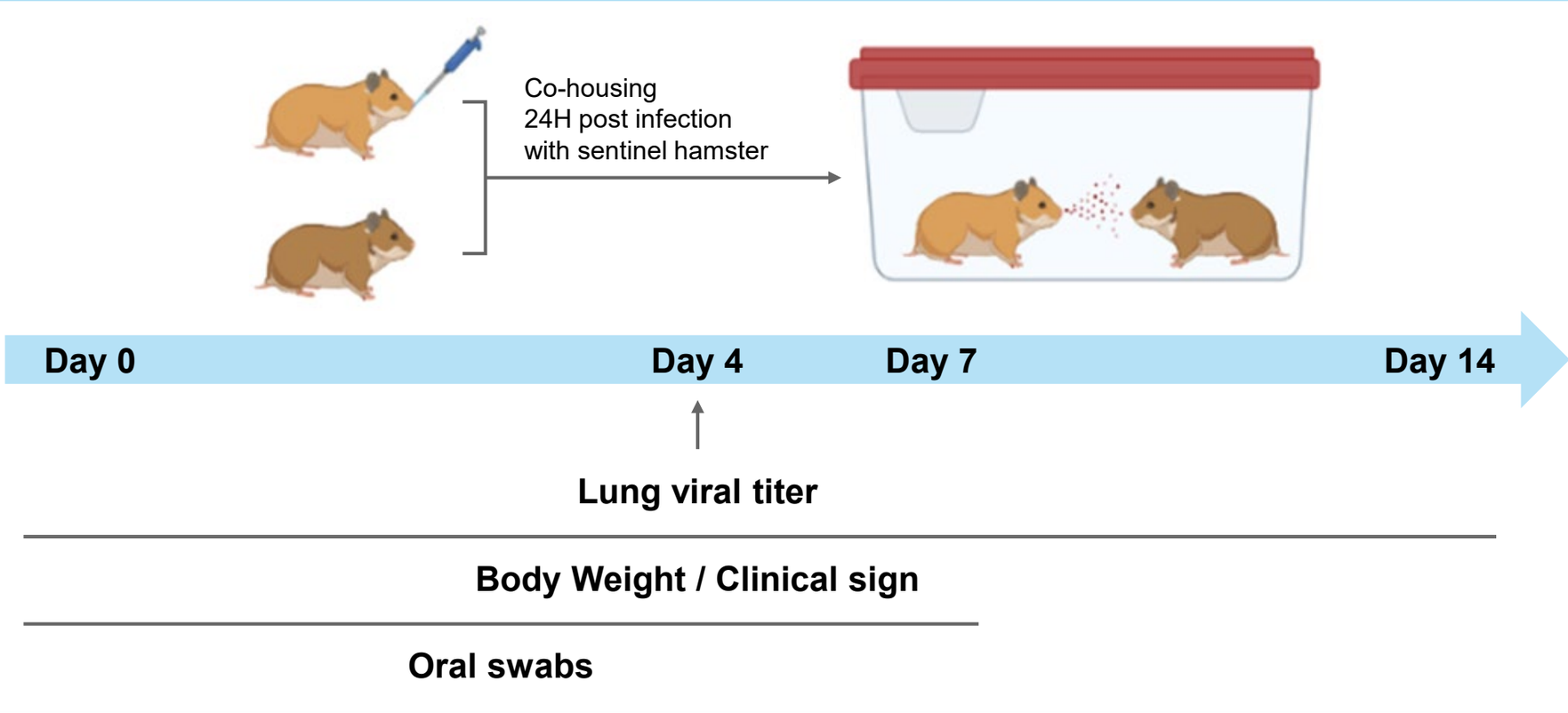


Virus Titre at Day 4



SARS-CoV-2 *in vivo* models

- Potential new endpoints
  - Virus titre in oral swabs
  - Cytokines by RT-qPCR
  - Lung histopathology and virus detection
- Potential new models
  - Transmission model
  - Different virus variants
- Potential mouse model
  - ACE2 transgenic mouse model



Anti-inflammatory assessment

Technology platforms

- Literature supports an important role of cytokine storms and hyper-inflammation, which cause an uncontrolled recruitment of macrophages and neutrophils to the infection site resulting in tissue damage. This effect is supported by the positive outcome observed with the treatment of IL-6 receptor antagonist Tocilizumab.
- A high inflammatory response could also contribute to tissue damage, including vasculitic lesions, blood vessel occlusion and infarctions (Felsenstein *et al,* 2020)
- Blocking the inflammatory response by inhibition of specific cytokine receptor signalling will be then of great value in reducing mortality (Zhang et. al., 2020)

PBMC isolation from healthy volunteers treated with potent T-cell activator

↓

Drug treatment

↓

Anti-inflammatory assessment

Cytokines/chemokines by Luminex (multiplex technology)

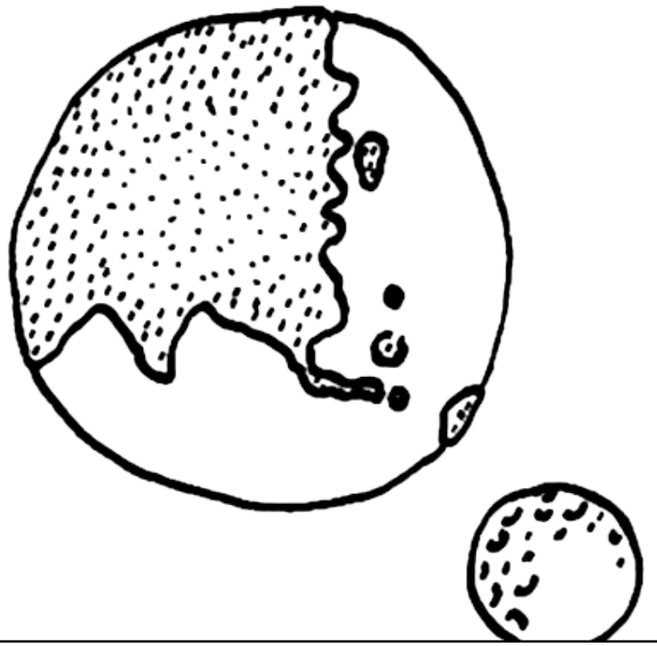
Immune activation of T and B cell by flow cytometry

Agenda

Evotec Overview

SARS-CoV-2 capabilities (*in vitro* and *in vivo*)

Summary



Virology at Evotec

Summary and future plans

- Virology has a continuously developing and broadening portfolio at Evotec
- Actively engaged in antiviral drug discovery
- Developing suite of *in vitro* and *in vivo* models
  - Inspired by and driven by collaborators
  - Set up of assays and models based on existing protocols or from the literature
  - Develop bespoke models based on TPP
  - Develop MOA strategy, e.g. isolation of resistant virus followed by sequencing
  - Examples:
    - *In vitro* assays for SARS-CoV-2, RSV, influenza virus, human rhinovirus, human coronavirus, hepatitis B virus
    - *In vivo* models for SARS-CoV-2, RSV, HRV and HBV

Translational approaches in industry

From unbiased to targeted testing

Unbiased biomarker discovery

- Genomics
- Transcriptomics
- Mass spectrometry-based proteomics and metabolomics
- Post-translational modifications (methyl, acetyl, phosphate, ubiquitin, glycosylation)
- Secretome analysis
- Immuno-phenotyping

Hypothesis testing

- *In vivo* models with high translational value (orthotopic, syngeneic, PDX, humanized etc.)
- *Ex vivo* drug treatment and/or analysis of both animal and human samples
- Preclinical imaging in rodents
- Exploration of prevalence in the context of pathology
- Evaluation of stratification, PD, toxicity, efficacy biomarkers
- Proposal for Phase 1 clinical trial

Clinical translation

Integration of different approaches to biomarker discovery best supports translational biology activities

Biomarker discovery approaches
PDX = patient derived xenograft

evotec

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