evotec

#RESEARCHNEVERSTOPS

Antiviral capabilities at Evotec

Focus on respiratory viruses and HBV

Agenda

Evotec Overview

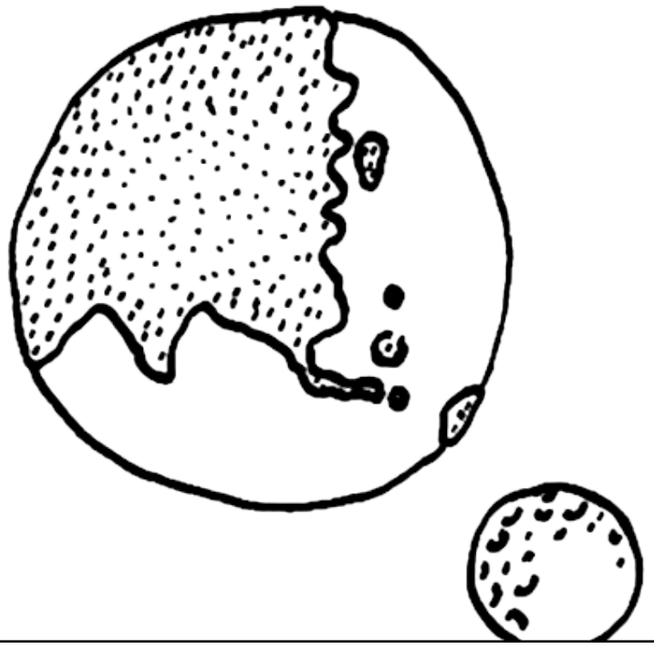
Respiratory virus capabilities (*in vitro* and *in vivo* models)

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

Hepatitis B virus capabilities

Integrated virology (Hit ID to PDC)

Summary



Fully integrated drug discovery and development

Opportunities to transform medical insights into products for patients

Sourcing novel ideas	Target ID/ Validation	Hit-Identification	Lead Optimisation	Pre-clinical	Phase I	Phase II	Phase III	Approval	Market
• Exploratory biology • Hit-finding technologies • Chemistry • DMPK • Sample Mgt.	• Ab Discovery • Biology • Design/Chemistry • DMPK • PK:PD	• Translational Biology • Design/Chemistry • DMPK/physical chem • Formulation • PK/PD & ADME • Safety	• Translational Biology • API Process Dev & Manufacturing • Formulation & Drug Product for clinical testing • Safety/MIST	• API and Drug Product commercial manufacturing					

Analytical Development, Quality control & Stability

- Interdisciplinary integration and seamless team working and evolution
- Innovation, high quality science, technology and problem-solving
- Knowledge and experience of successful practitioners: **Ideation to the Clinic and beyond**
- Under “ONE Evotec” roof offering, unique breadth, capacity, knowhow, track record
- Operational excellence to drive rapid progress and successful outcomes

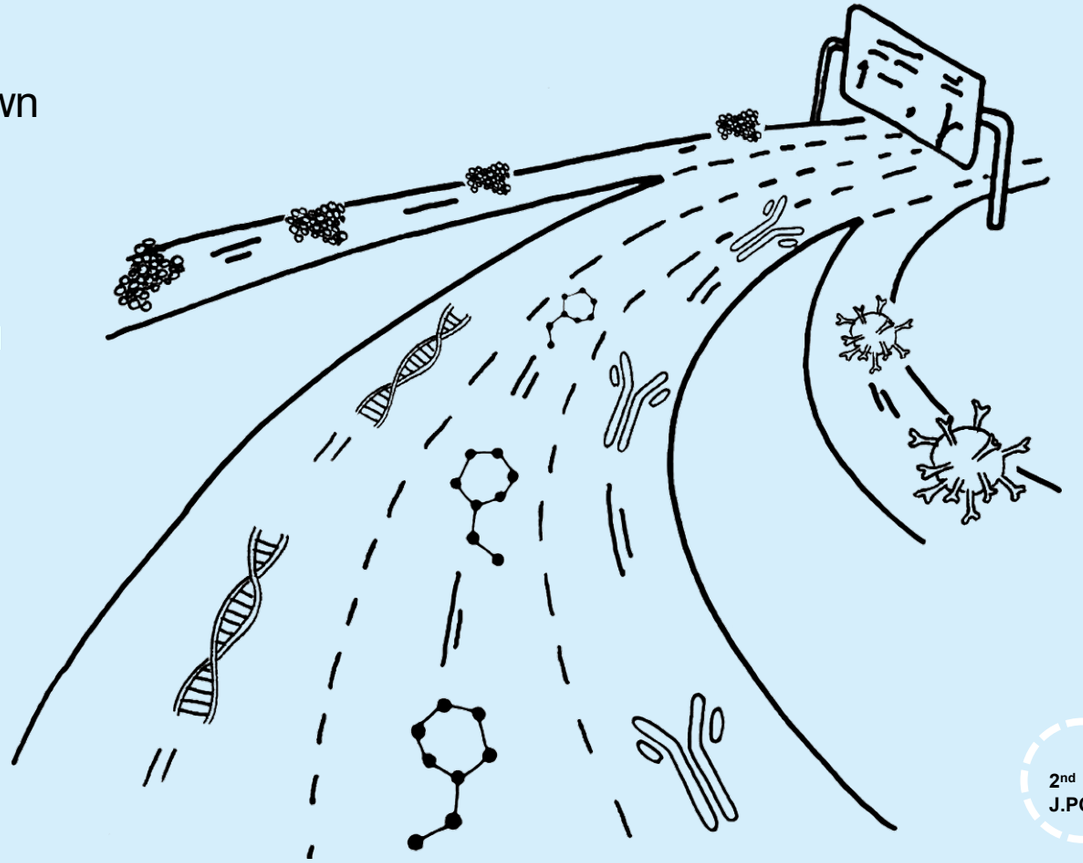
One platform – more efficiency, better precision, higher speed

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

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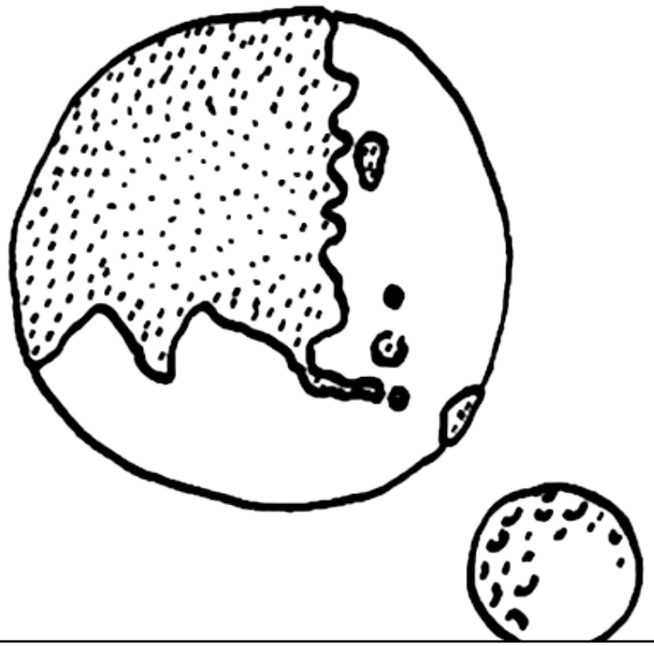


Evotec Viral Screening Capabilities

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

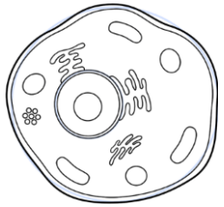
BSL3

BSL3

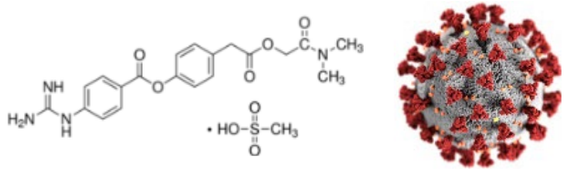
Live virus compound testing for respiratory viruses

Screening and profiling assays under BSL2/ BSL3 containment

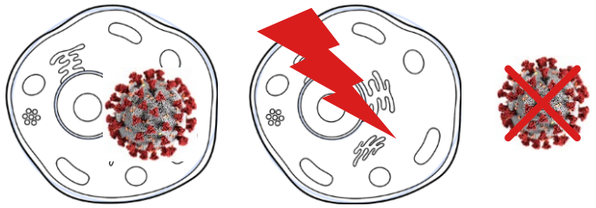
Seed Cells



Add Compound + Virus



Measure Cell Viability

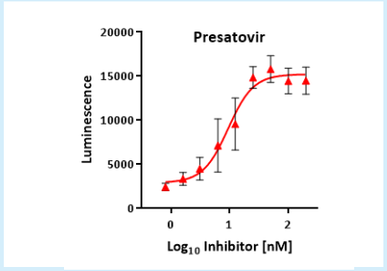


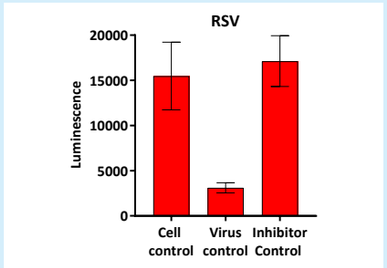
- Routine cellular screening assays against respiratory viruses
  - Standard assay set up:
  - Cells seeded
  - Infected with virus, treated with compound
  - Incubation between 24 and 96h depending on viral growth
  - Cell viability measured by Viral Toxglo™
- Assay can be performed in 96-well or 384-well format
- All plates containing uninfected cell control, virus control and inhibitor control
- Cell lines with different properties can be selected depending on intended target
  - ToxGlo assay restricted to cell lines where virus causes cytopathic effect
- Alternative/ additional endpoints
  - ELISA against viral protein or RT-qPCR in cell lines where virus does not cause CPE
  - Evaluation of viral progeny by plaque assay or RT-qPCR
- 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

Live virus compound testing for respiratory viruses

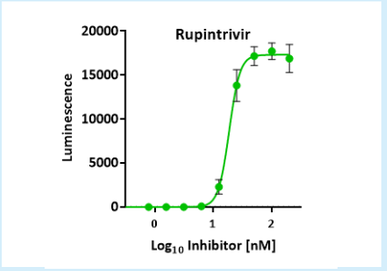
- ToxGlo Assay
  - SARS-CoV2
  - Human coronavirus strains 229E, OC43 and NL63
  - Respiratory syncytial virus A2 and clinical isolates
  - Human rhinovirus 14 and 16
  - Parainfluenza virus 3
  - Human metapneumovirus (in development)

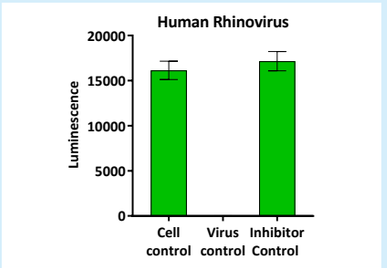
RSV



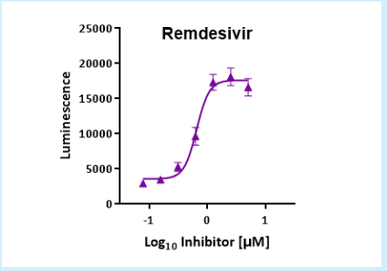


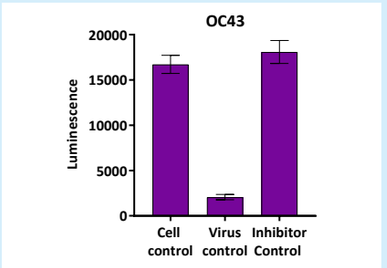
HRV



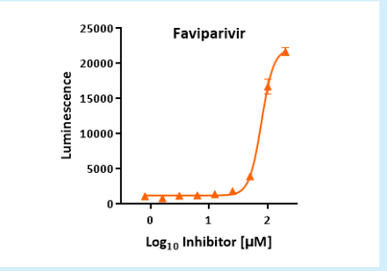


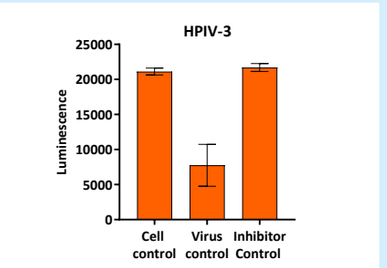
Human coronavirus OC43





PIV

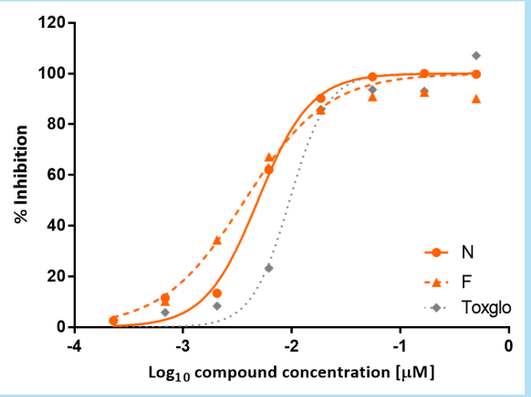


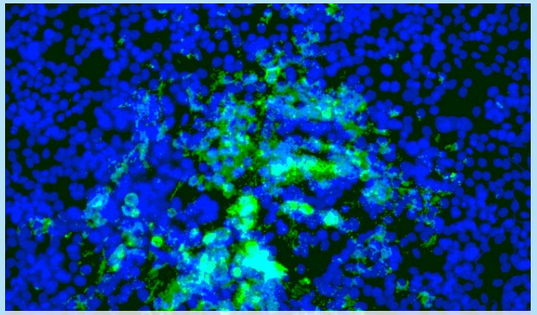


RSV *in vitro* assays

RSV viral culture in HEp2 cells suitable for low to medium throughput assays, other cell lines are available for infection

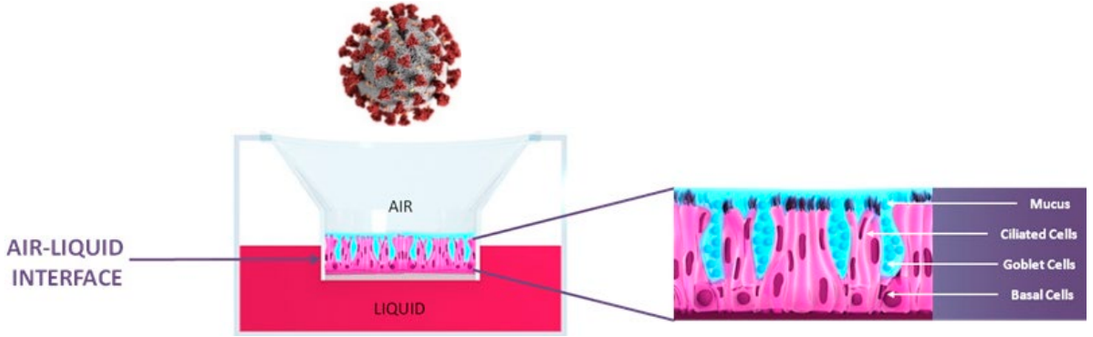
- Viral ToxGlo™ screening assay
  - Single step assay measuring metabolic activity
  - Medium throughput screening and dose response
  - Also suitable for cytotoxicity counter screens
- RSV plaque assay
  - Quantification and validation of viral stocks and viral burden in tissue, e.g. as read-out for *in vivo* studies
  - Generation of resistant virus
  - Mechanistic studies
- RSV ELISA
  - Quantification of virus specific antibodies
  - Quantification of virus via viral proteins
- RSV microneutralization assay
  - Quantification of virus specific neutralizing antibodies
- Several RSV-A and RSV-B strains available
  - Demonstration of broad antiviral effect





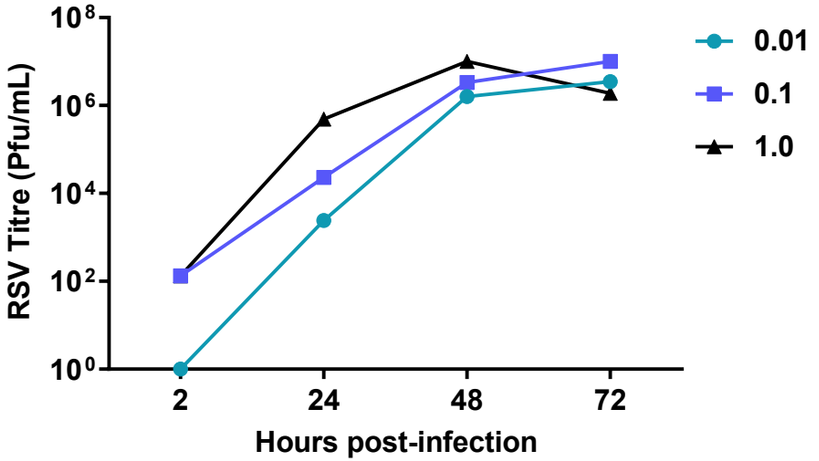
RSV A2 virus infected HEp2 cells stained for RSV F protein (green) and DAPI (blue)

Human airway epithelial assay



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

Timecourse of RSV infection at different multiplicity of infection

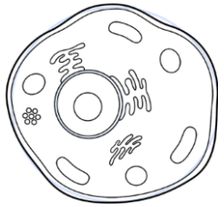


- MucilAir™ is a human *in vitro* model, representing the upper airway epithelia containing beating cilia, goblet and basal cells
- The model is a highly relevant and useful tool to address pharmacology, toxicology and biology demands, in particular for cellular targets
- Assay design has been used for Respiratory Syncytial Virus (RSV) and can be adapted for other viruses
- Endpoint is CPE as determined by plaque assay with lower throughput

Influenza virus

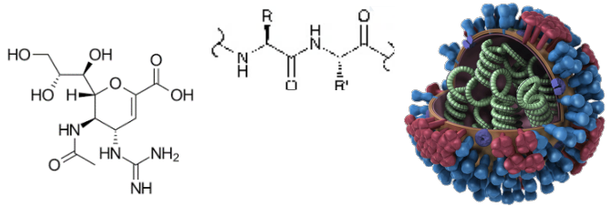
Influenza Assay Protocol and Validation

Seed MDCK cells



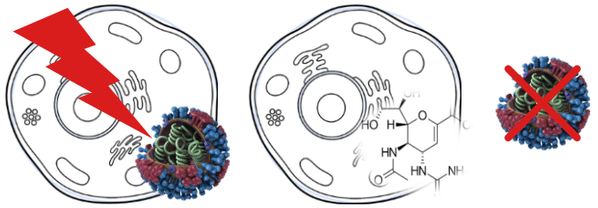
24h

Add Compound + TPCK-Trypsin + Virus



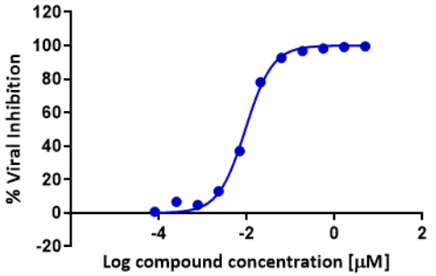
72h

Measure virus neuraminidase activity

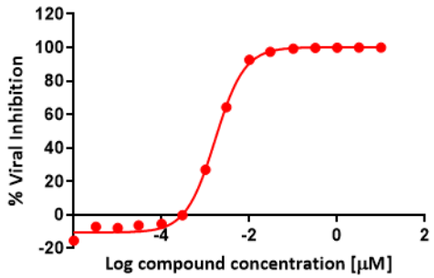


- A549 or MDCK cells are available for influenza virus assay
- Infected with Influenza A virus (FluA Brisbane/59/2007, H1N1)
- Cells treated with anti-viral test articles in dilution series in 96 or 384-well format
- Virus replication measured by virus neuraminidase activity readout (AVINA assay)
- All plates containing uninfected cell control, virus control and inhibitor control (Zanamivir)
- Virus inhibition calculated relative to virus control, negative signal for inhibition
- Cytotoxicity testing in the respective infected cell line performed in parallel to identify potential false positives

Zanamivir – A549 cells



Zanamivir – MDCK cells



Influenza virus

Influenza virus PA endonuclease assay

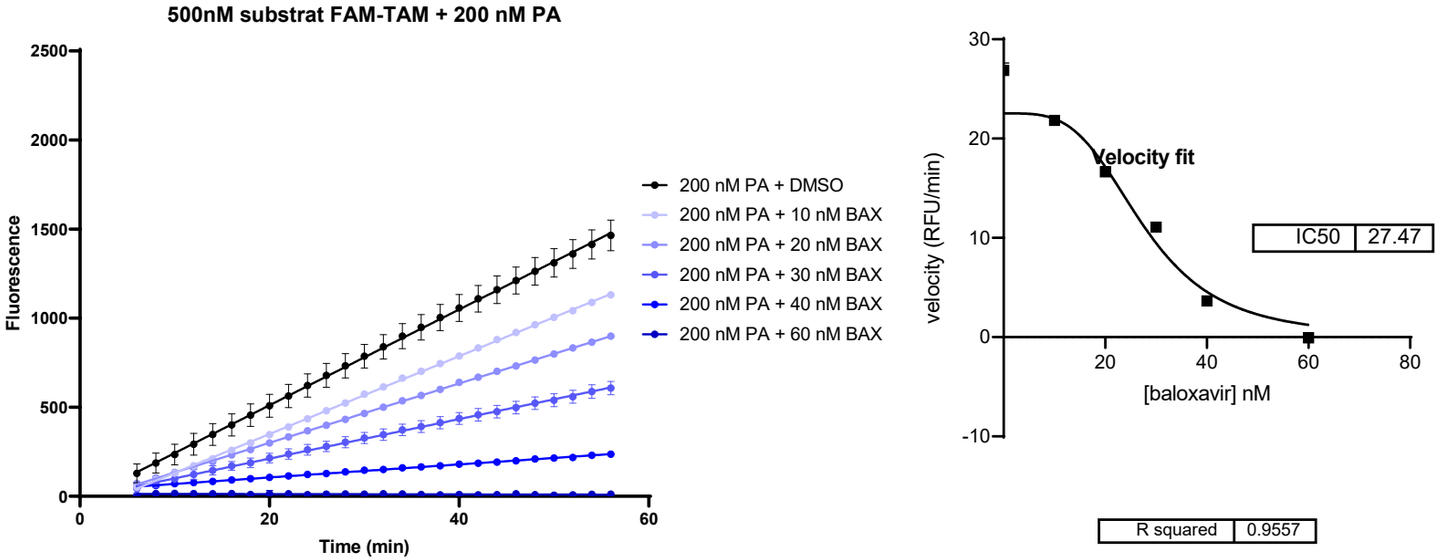
Principle

FRET based assay for detection of PA endonuclease activity via fluorescence.

Sequence - PA-substrate

5'- /56-FAM/AAT CGC AGG CAG CAC TC/36-TAMSp/ -3'

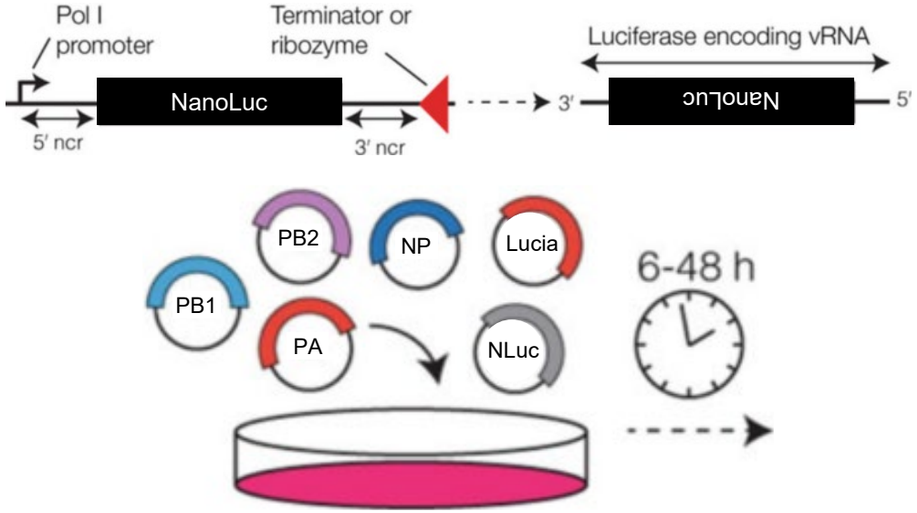
Effect of Baloxavir

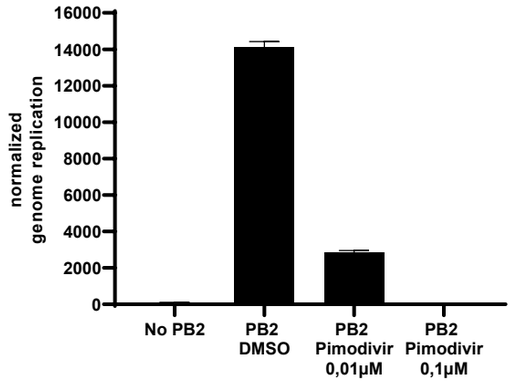


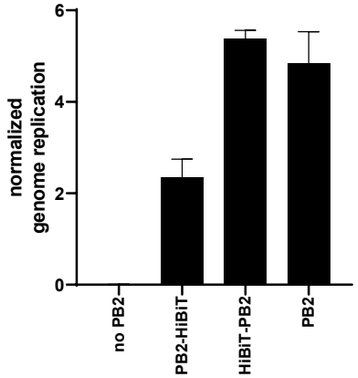
Te Velthuis, A. J. W., et al. (2018). "Assays to Measure the Activity of Influenza Virus Polymerase." Methods Mol Biol **1836: 343-374**.
Noble, E., et al. (2012). "Endonuclease substrate selectivity characterized with full-length PA of influenza A virus polymerase." Virology **433(1): 27-34**.

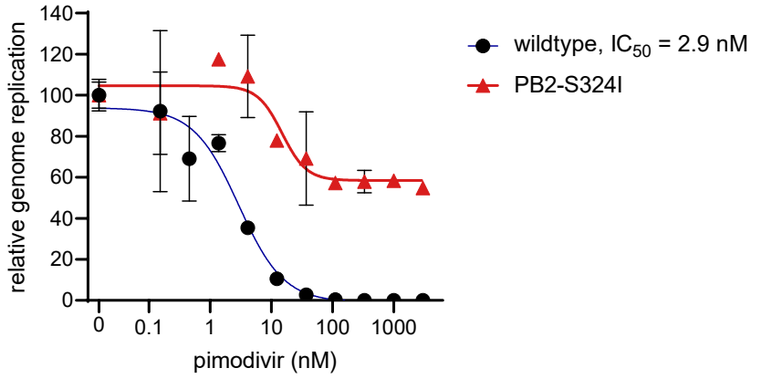
Influenza minigenome assay

- Intracellular NanoLuciferase (genome replication)
- Secreted Lucia in supernatants (normalization)
- Pimodivir as control inhibitor
- Test resistant mutants









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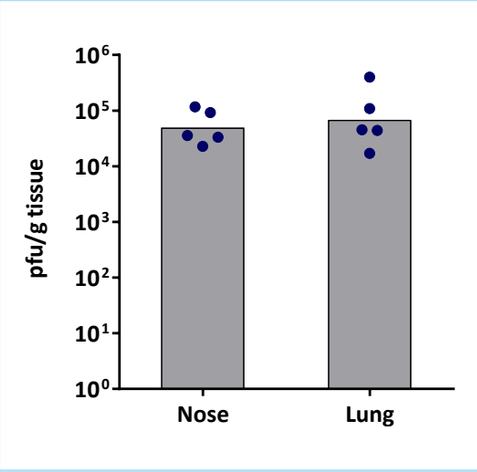
RSV *in vivo* model

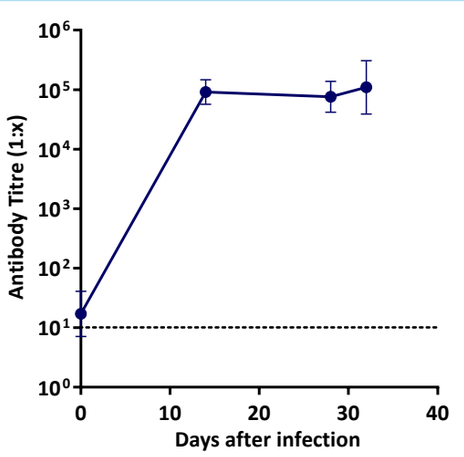
Cotton Rat Infection Model

- Gold standard model for development of RSV inhibitors
- Cotton rats are not standard laboratory animals
  - Animals handled in specific manner to reduce stress including cage changes and all procedures
  - Most procedures performed under brief isofluorane anaesthesia
- Model Read-Outs that have been validated
  - Viral load measured 4 days post intranasal infection in nose and lung tissue by plaque assay; improved tissue extraction method for quicker processing
  - Antibody titre via ELISA
  - Neutralising antibodies in neutralisation assay
  - Immunohistochemistry
  - qPCR for viral load
- Results
  - Burden high until day 4 post infection, cleared by day 6
  - High levels of RSV specific antibodies throughout course of infection
  - Antibodies are able to neutralise infection
- Model has been used in several projects for vaccination and treatment studies

Viral burden



RSV specific antibodies

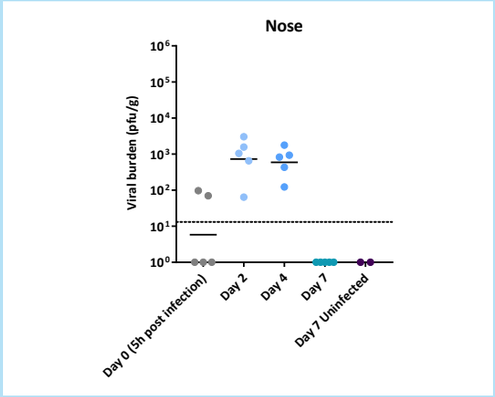


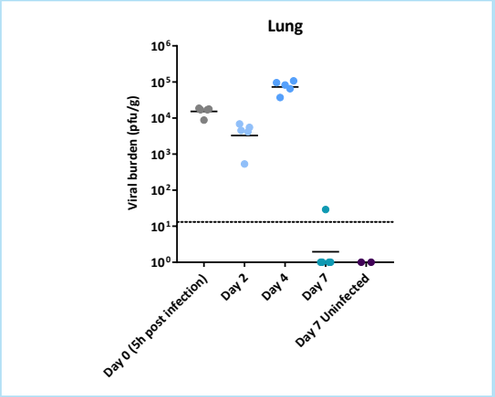
RSV *in vivo* model

Mouse Infection Model

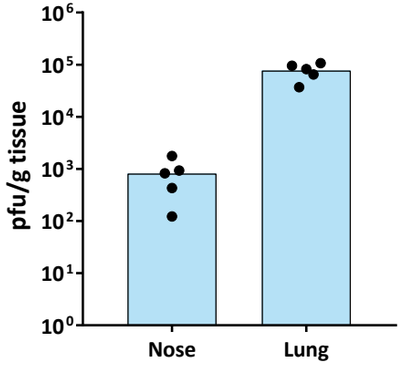
- Balb/c mouse model widely used to study RSV immunopathology
  - Semi-permissive to the virus
  - Similar viral growth kinetic to the cotton rats; viral titre peaks at day four and declines to undetectable at day seven
  - More cost effective compared to the cotton rat model
  - Intranasal infection, RSV-A2 4.25 x 10^5 pfu/animal
- Validated model endpoints
  - Viral load in nasal tissue by plaque assay (24- and 96-well format)
  - Viral load in lung homogenate by plaque assay
  - qPCR for viral load
  - Cytokine levels determined by ELISA
- Results
  - Consistently higher burden in the lung than in the nose
  - RSV detected within 5 hours of infection
  - Infection cleared by day 7
  - Burden data tight and reproducible

Time course of infection

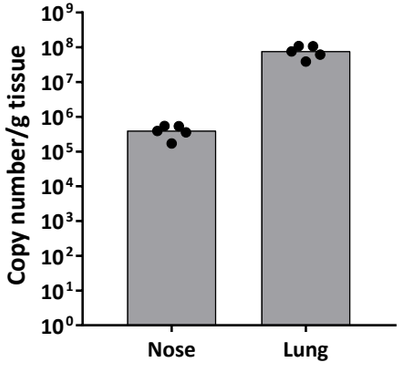




Plaque assay



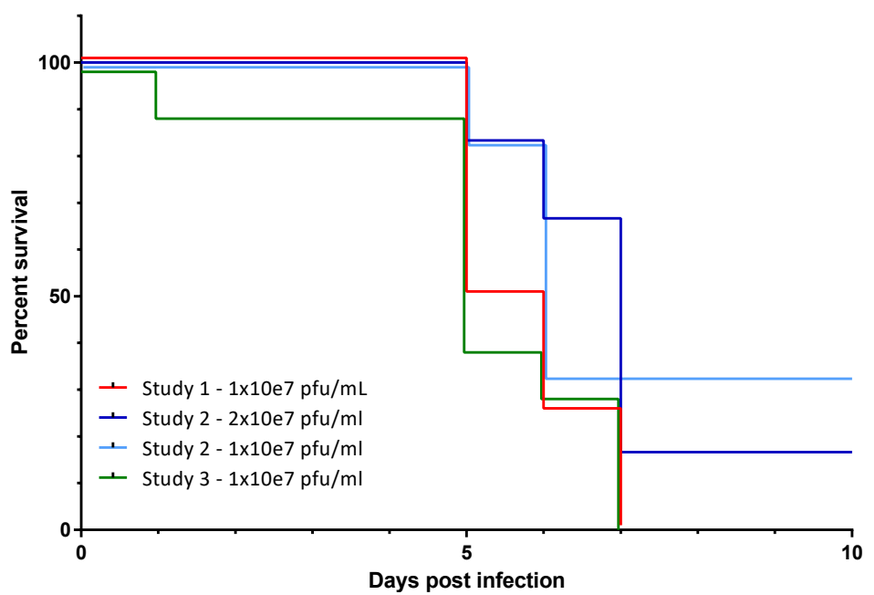
RT-qPCR



HRV *in vivo* model

- Coxsackie virus neonate model (surrogate model)
  - Not a standard model
  - CD1 mice time mated
  - Neonates (1-5 days) infected by intraperitoneal inoculation with Coxsackie virus A10
  - Survival as endpoint
  - Suitable for treatment studies (oral or SC dosing) up to 4 times daily
- Mouse HRV infection model (can be developed)
  - HRV-1B infection of wild-type mice
  - Viral replication attenuated and infection is cleared within 2 days
  - Animals do not develop significant symptoms, beside mild signs of inflammation of the lungs
  - Endpoints: viral burden in lung tissue, inflammation markers including immune cell infiltration of the lungs

Coxsackie virus neonate model



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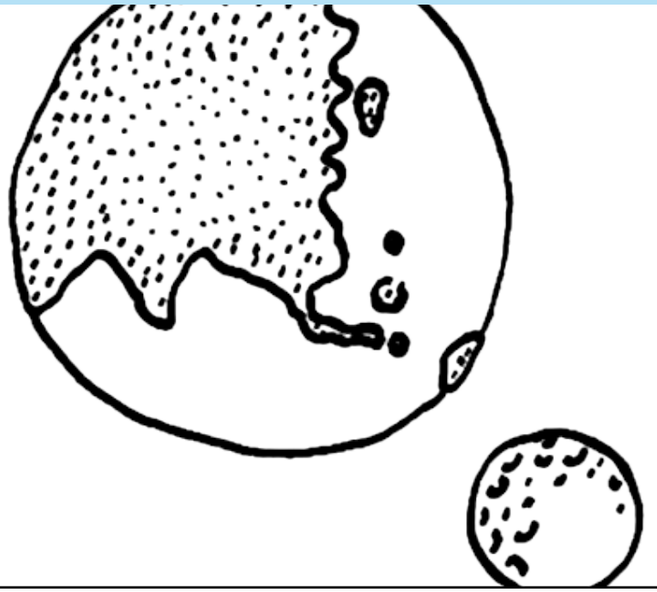
SARS-CoV-2 capabilities (*in vitro* and *in vivo*)

Hepatitis B virus capabilities

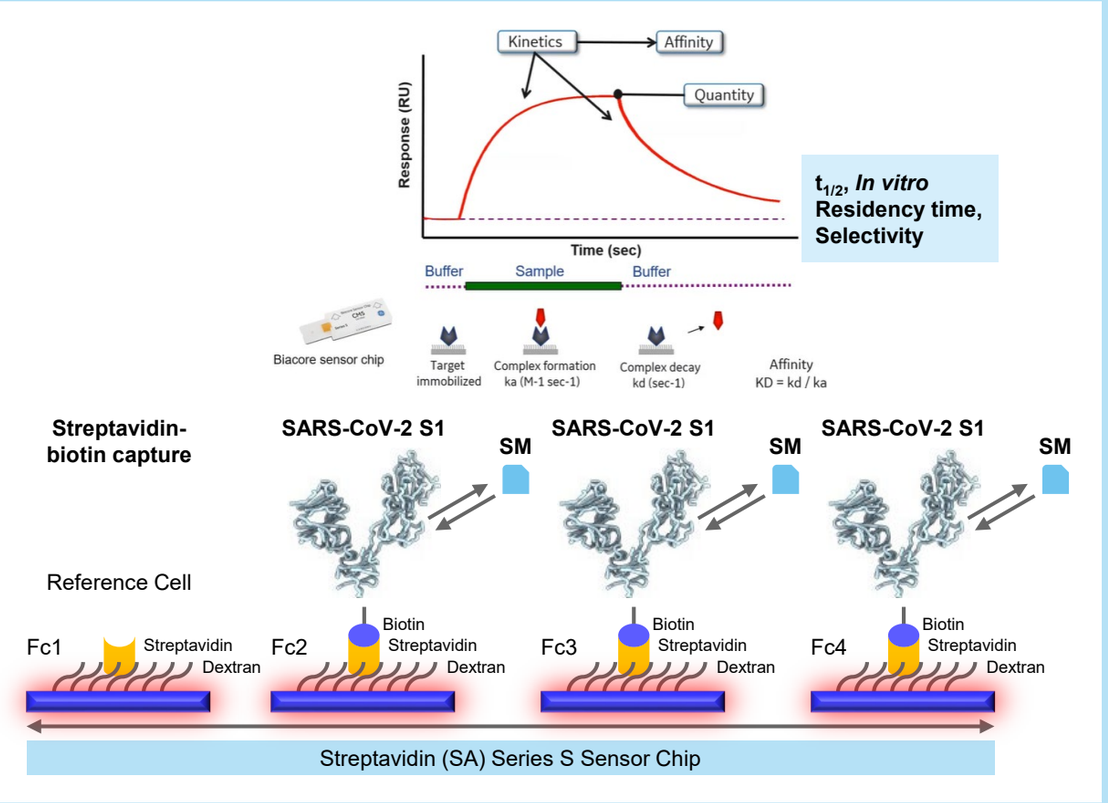
Integrated virology (Hit ID to PDC)

Summary



SARS-CoV-2 *in vitro* pharmacology assays

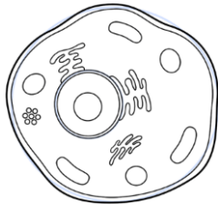
- Background
  - Coronavirus uses spike glycoproteins found on the envelope to gain entry into the cells
  - Spike proteins are homotrimers comprising S1 and S2 subunits
  - SARS-CoV-2-Receptor Binding Domain (RBD) binds ACE2 receptor with high affinity, triggering S2 to divide from S1 and transit to 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



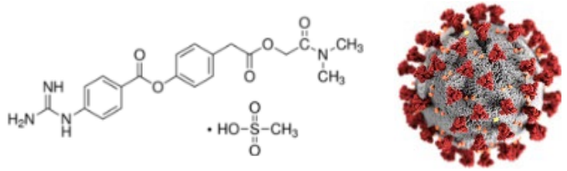
SARS-CoV-2 infection model

Screening and profiling assays under BSL3 containment

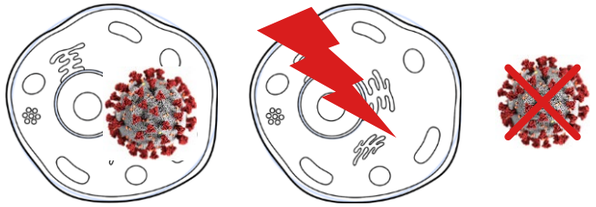
Seed Cells



Add Compound + Virus



Measure Cell Viability

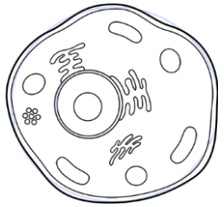


- Routine cellular screening assays against SARS-CoV-2 strains
  - 9 strains established: Alpha, Beta, Delta, Gamma variants
  - 29 additional strains available upon request, including Mu and Lambda
- Standard assay set up:
  - Cells seeded
  - Infected with virus, treated with compound
  - Incubation for 72h
  - Cell viability measured by CellTiter Glo™
- Assay is performed in 96-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 (Vero E6) for CellTiter Glo™ assay
  - RT-qPCR can be used also in cell lines where virus does not cause CPE (Calu-3, A549-hACE2 and A549-hACE2/hTMPRSS2)
- Cytotoxicity testing in uninfected cell lines performed in parallel to identify potential false negatives

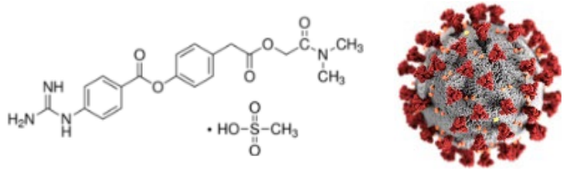
SARS-CoV-2 infection model

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

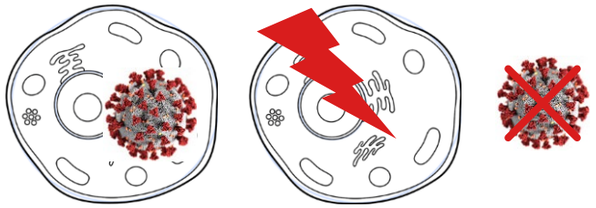
Seed Cells



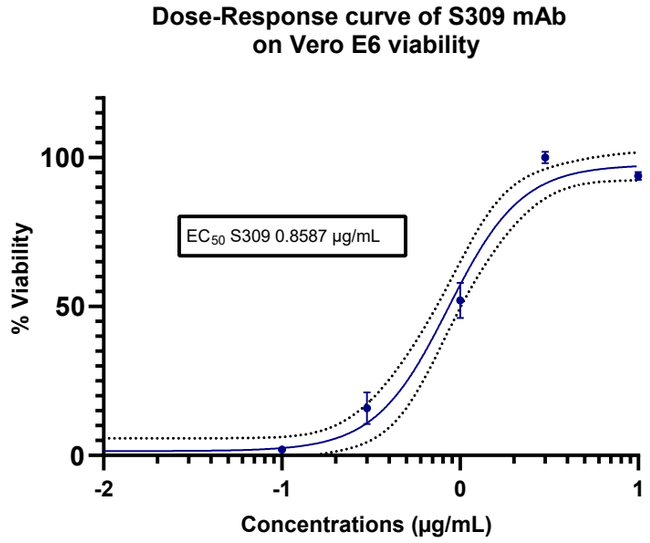
Add Compound + Virus

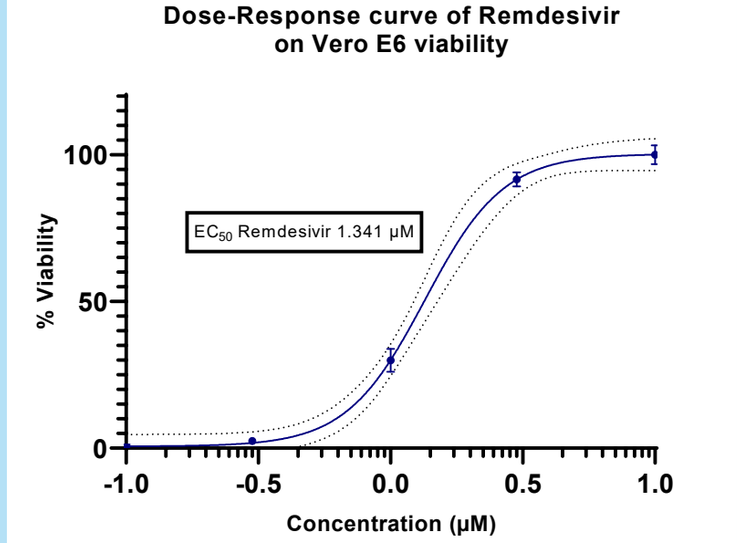


Measure Cell Viability



SARS-CoV-2 infection





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

Several SARS-CoV-2 variants are available

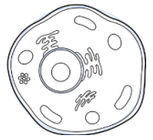
Virus (original stock)	Lineage	GISAID clade	Comments
SARS-CoV-2 Germany/BavPat1/2020	B	G	–
SARS-CoV-2 USA-WA1/2020	A	S	–
SARS-CoV-2 Chile/Santiago_op4d1/2020	A.2	S	–
SARS-CoV-2 England/02/2020	A	S	–
SARS-CoV-2 hCoV-19/England/204820464/2020	B.1.1.7	GR	Alpha
SARS-CoV-2 hCoV-19/South Africa/KRISP-EC-K005321/2020	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

SARS-CoV-2 infection model

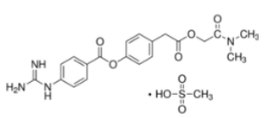
RT-qPCR analysis

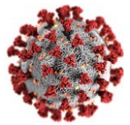
Seed Cells



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

Add Com-pound + Virus





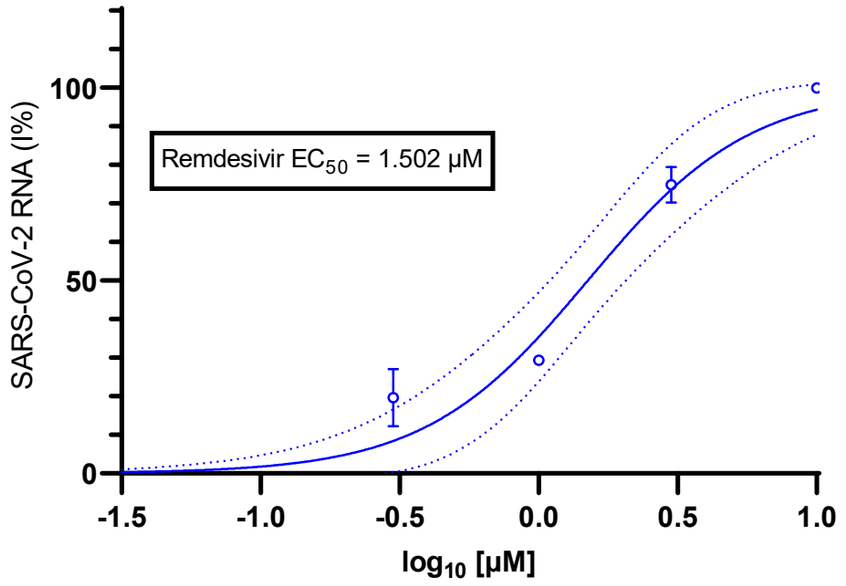
3d incubation

Cell lysis and RNA extraction

RT-qPCR

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

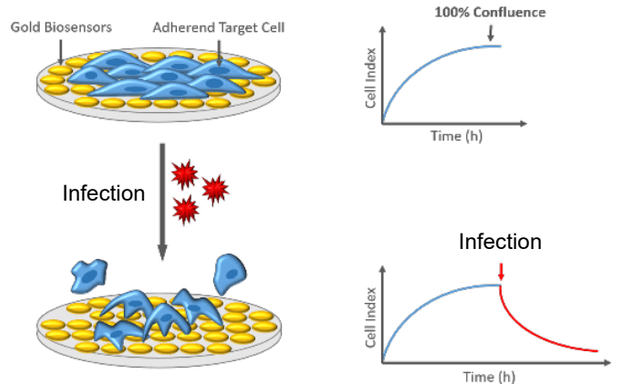
Effect of Remdesivir on SARS-CoV-2 RNA quantification by RT-qPCR in cell lysates



SARS-CoV-2 infection model

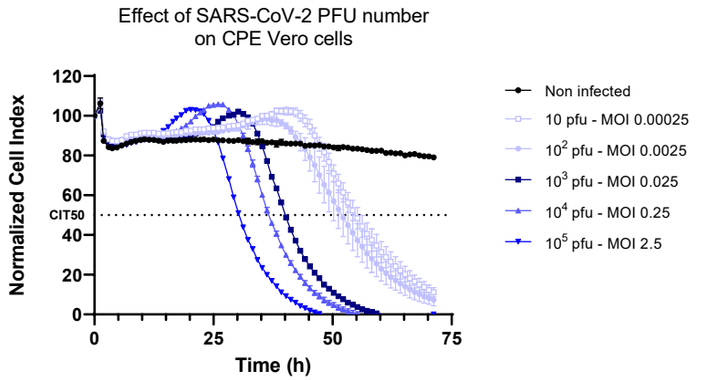
xCELLigence – Vero E6

xCELLigence principle



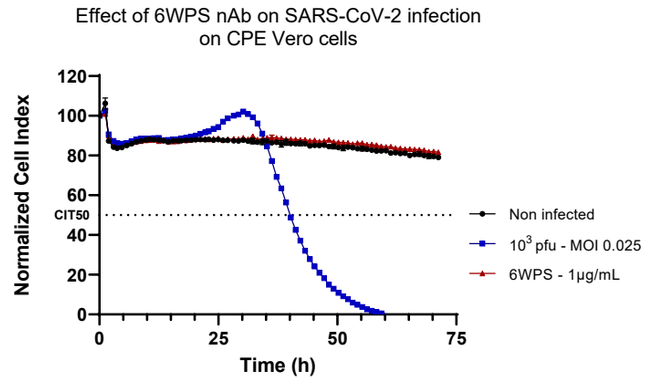
- Vero cells in 96w E-plate – 4 x10^4 cells/well
- Strain SARS-CoV-2 USA-WA1/2020
- Tested number of PFU: from 10 to 10^6
- 72h infection
- Detection of electrical impedance every 15 min

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 all variants of concern available including

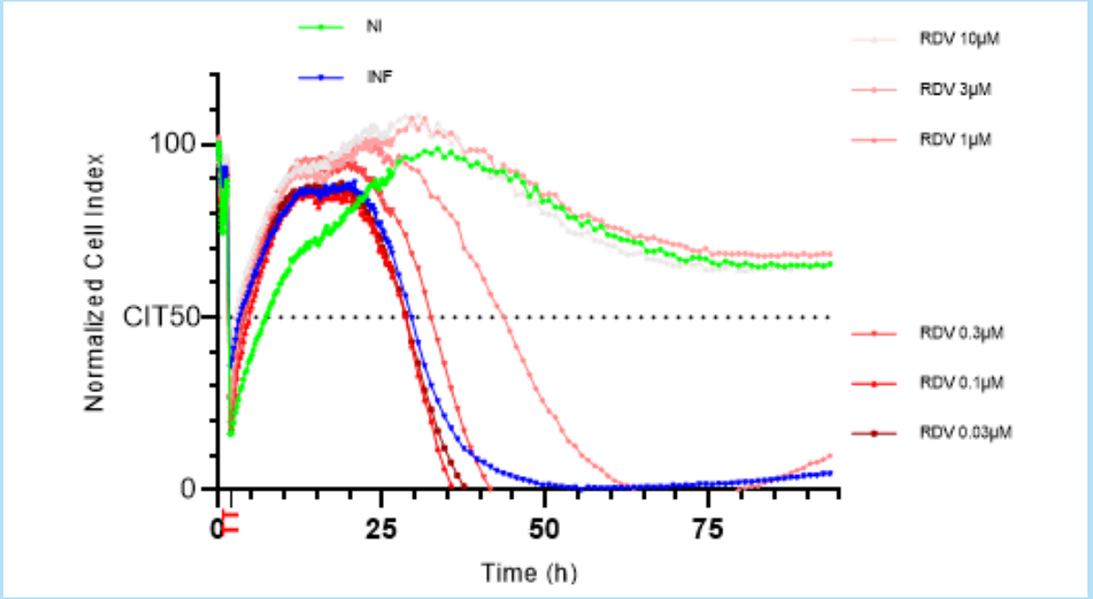
- Delta
- Omicron

PFU 2-fold difference in CIT_{50}

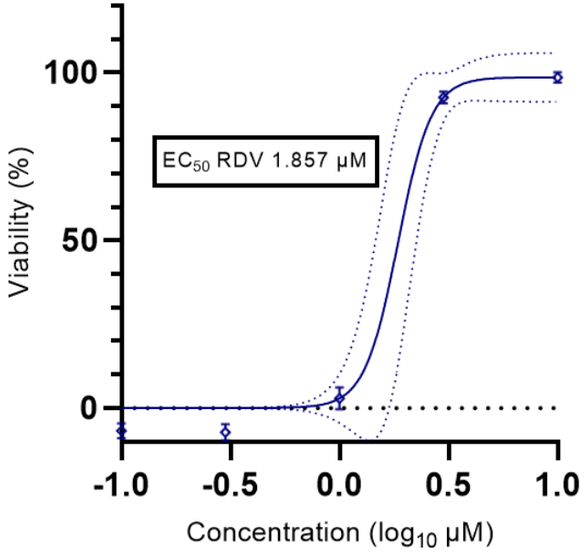
SARS-CoV-2 infection model

xCELLigence – Vero E6

Effect of Remdesivir



Determination of EC_{50}

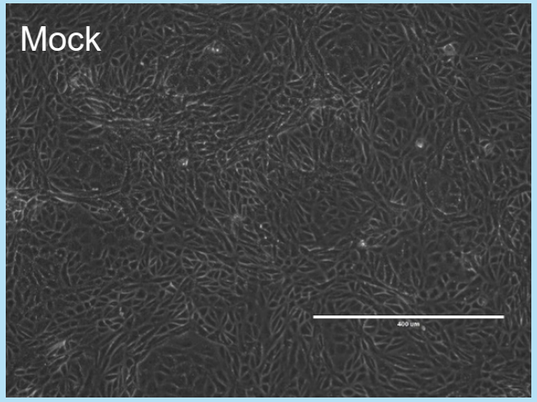


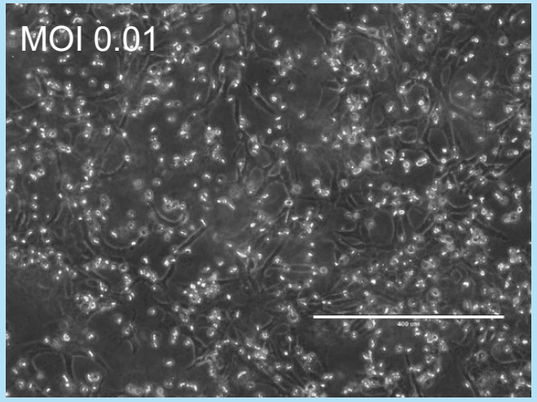
Remdesivir inhibits SARS-CoV-2 infection in a dose dependent manner, that allows determination of EC_{50}

Plaque-forming Assay

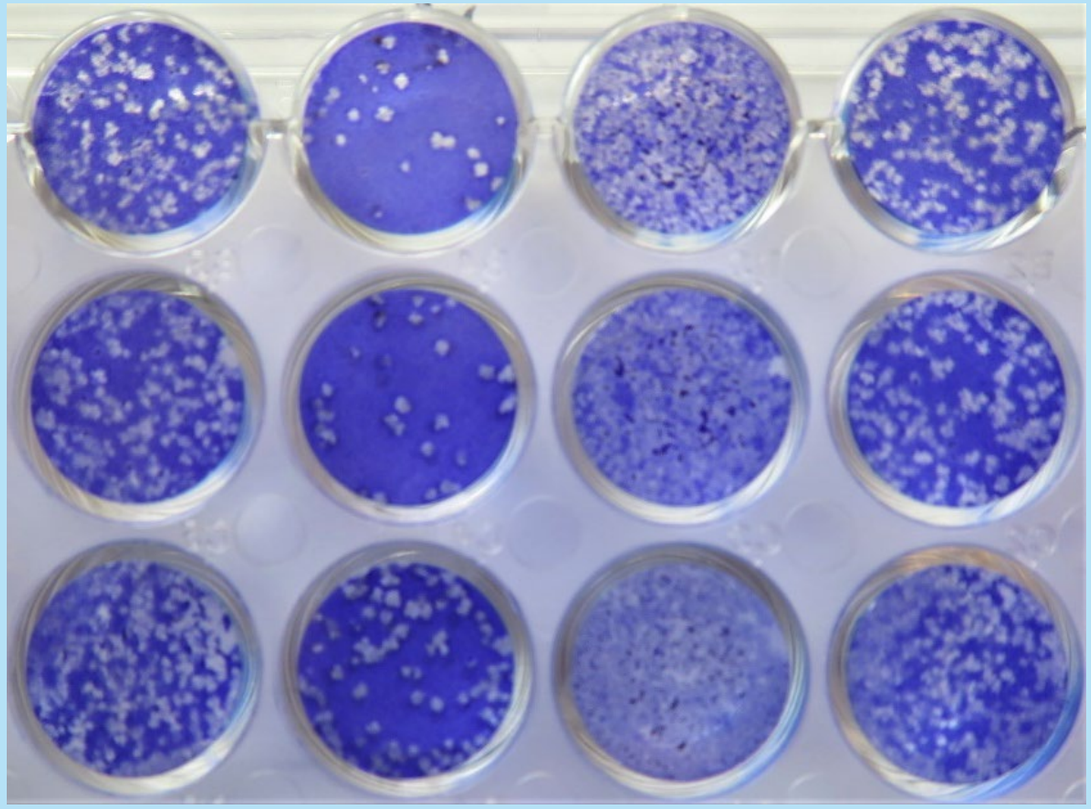
Titration of SARS-CoV-2

- 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



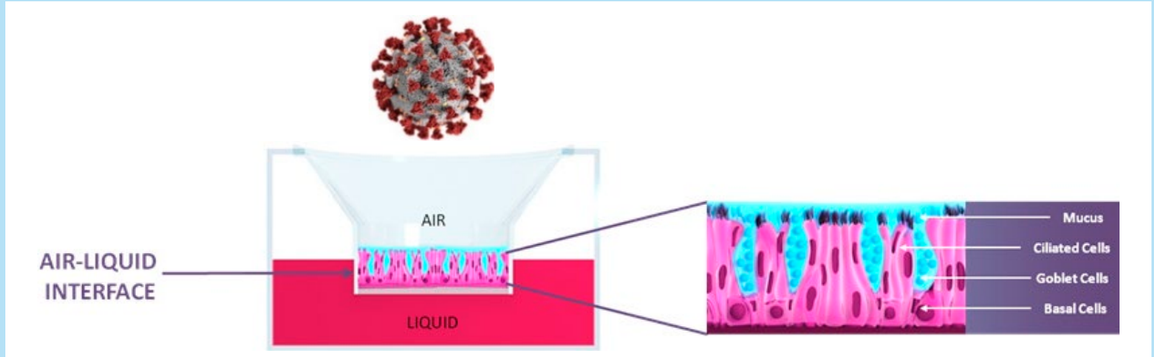


Virus-induced CPE

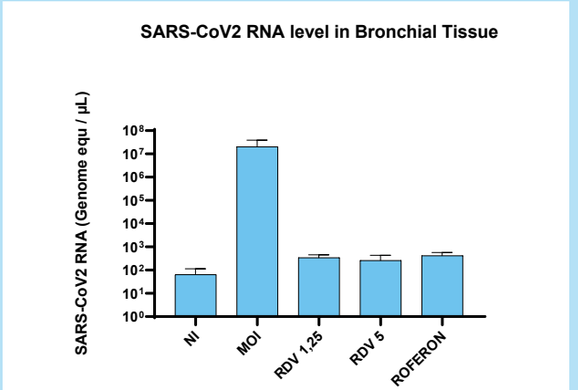


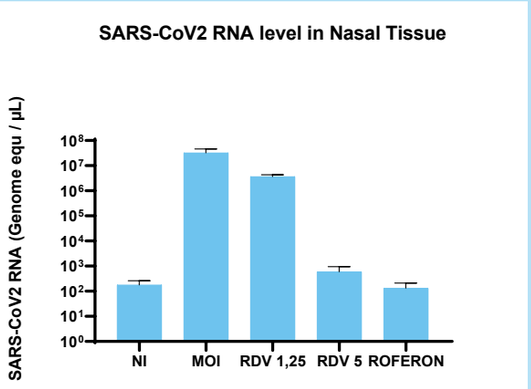
Human airway epithelial assay

Initial data on SARS-Cov-2



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

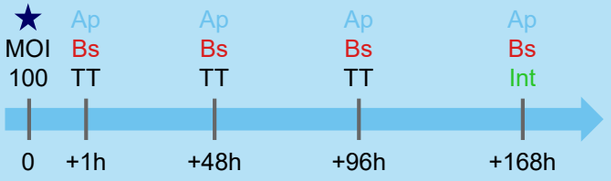




- MucilAir™ is a human *in vitro* model, representing the upper airway epithelia containing beating cilia, goblet and basal cells
- The model is a highly relevant and useful tool to address pharmacology, toxicology and biology demands
- Endpoint is viral load quantified by RT-PCR on basal, apical and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

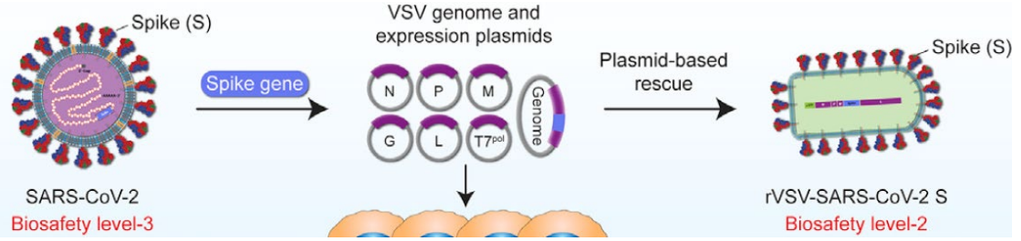
Int Intracellular viral quantification

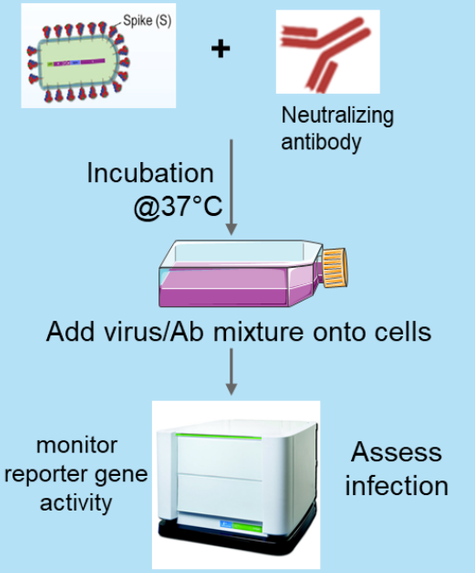
SARS-CoV-2-Spike pseudotyped infection

Pseudotyped viruses with different variants are available

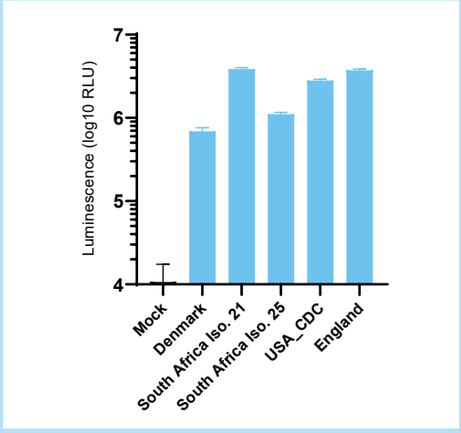
Generation of VSV bearing SARS-CoV-2 spike gene (rVSV-SARS-CoV-2)

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

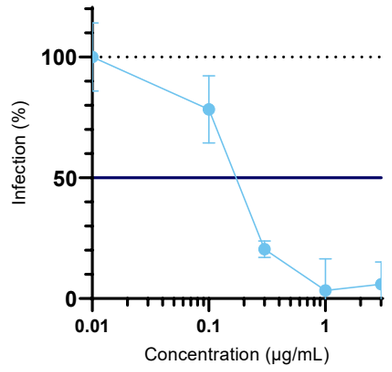




Generation of VSV bearing SARS-CoV-2 spike variants



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

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

↓

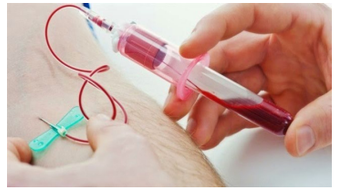
Anti-inflammatory assessment

Cytokines/chemokines by Luminex (multiplex technology)

Immune activation of T and B cell by flow cytometry

Inflammatory cytokines release from human whole blood cells *ex vivo*

Assessment of potential CRS

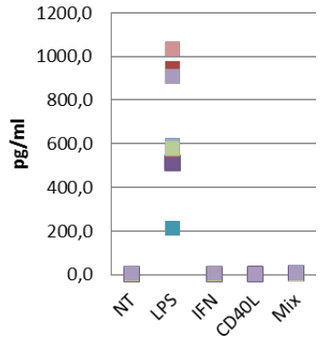


Whole blood cells from 10 donors

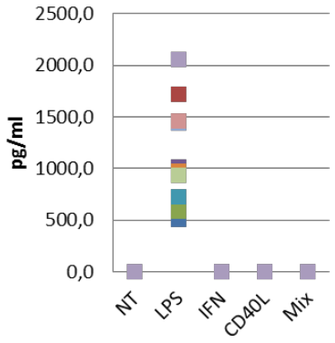
- **Positive control: LPS, 10ng/ml**
- **Stimulation: 24h**

Cytokines analysed: TNFα, IL1β, IL6, IL8, IFNγ, IL10, IL12/p40, IL2 and CXCL10

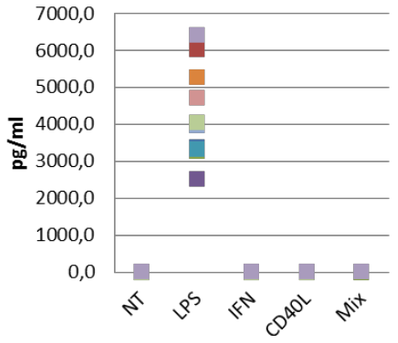
TNFα



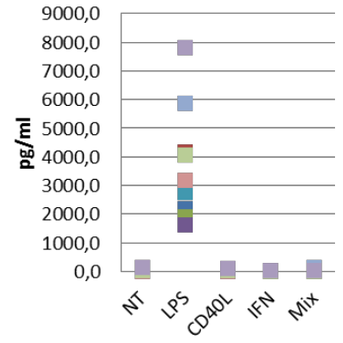
IL1β



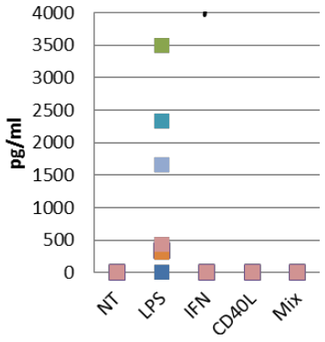
IL6



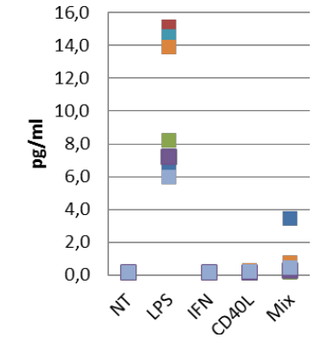
IL8



IFNγ



IL10



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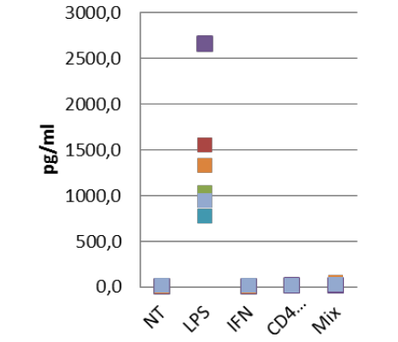
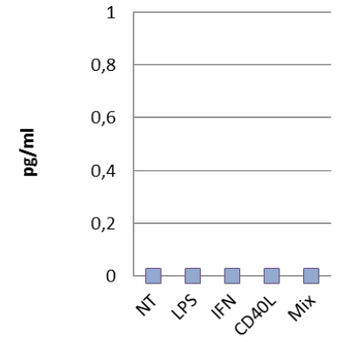


IL2

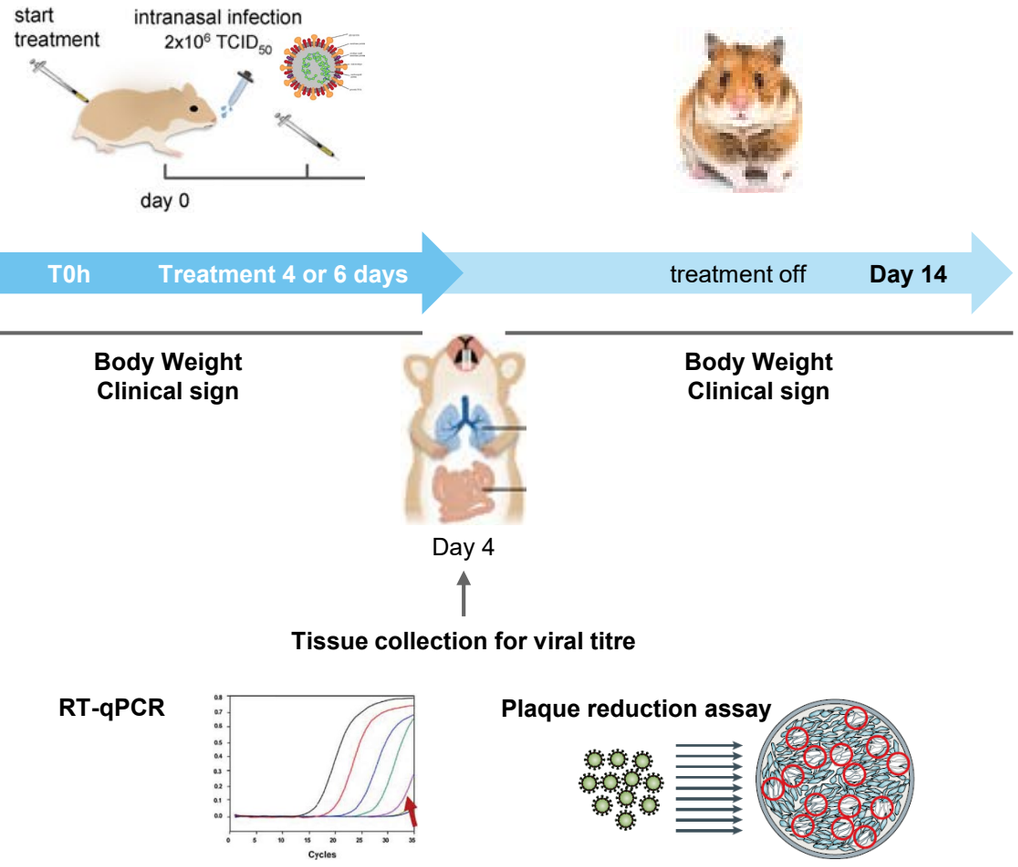


CRS: Cytokine Release Syndrome

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

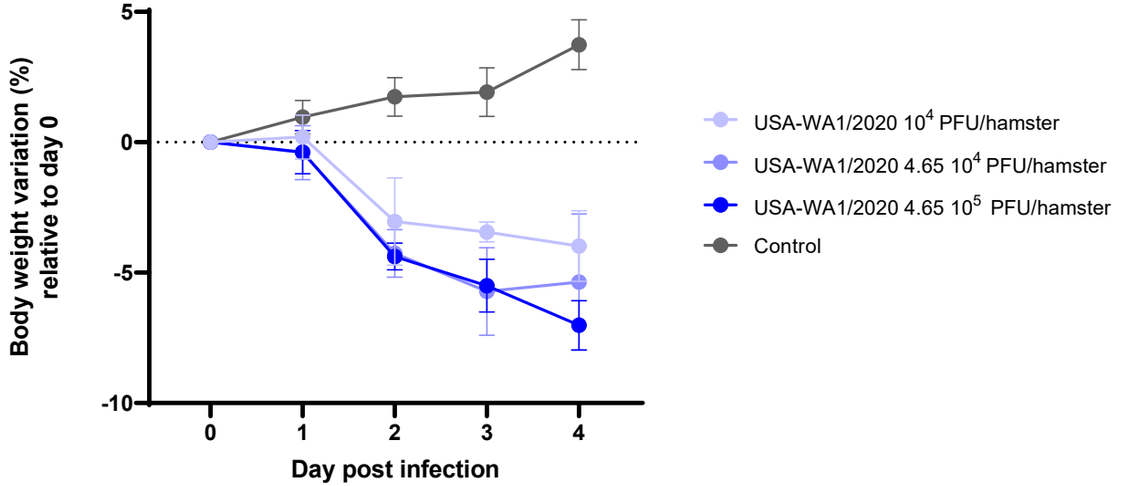
Additional endpoints

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

SARS-CoV-2 Hamster model development

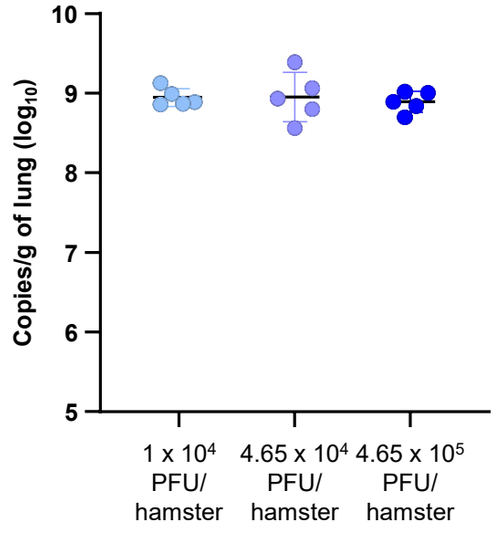
Examples of Study Endpoints

Body weight

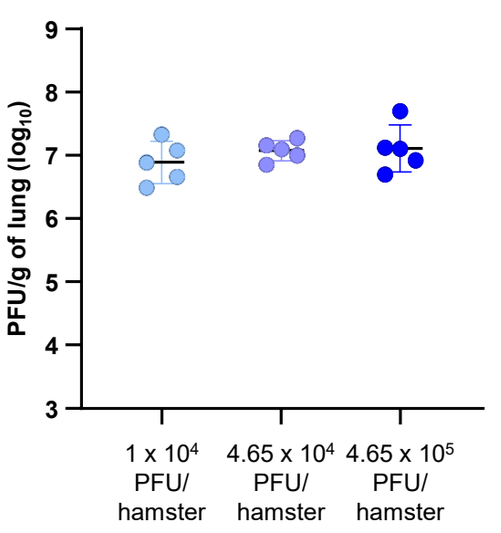


Virus strain USA-WA1/2020,
Body weight loss induced by SARS-CoV-2 Infection

RT-qPCR



Plaque assay



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

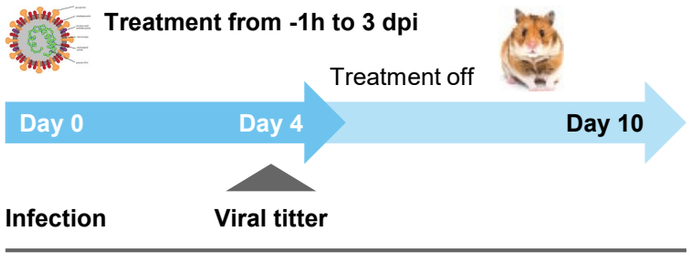
Average ± SD

SARS-CoV-2 Hamster model

Validation with reference compound

Infection

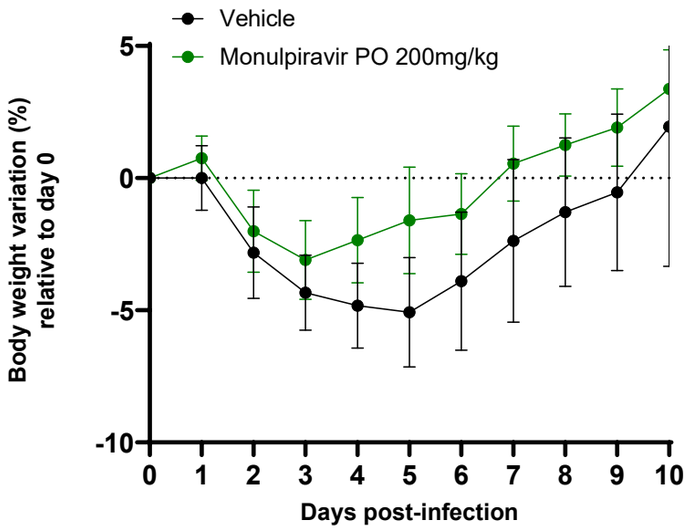
- SARS-CoV-2 USA-WA1/2020



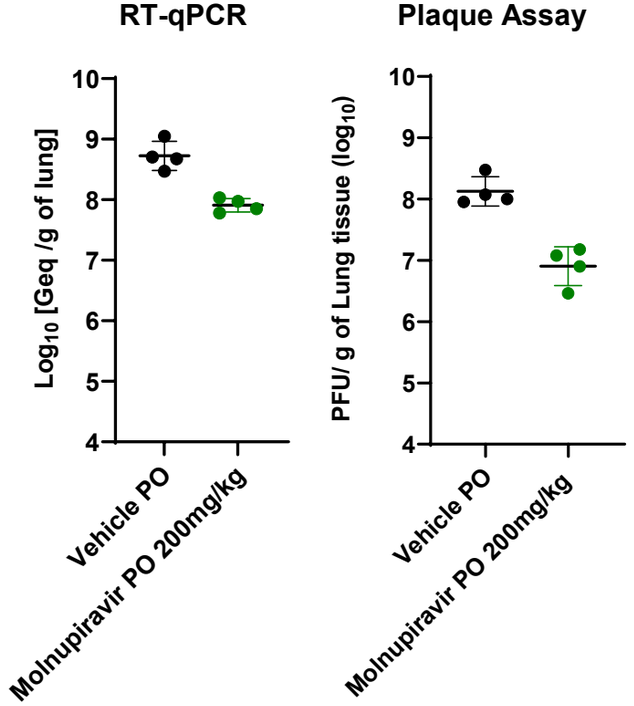
Body Weight / Clinical sign

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

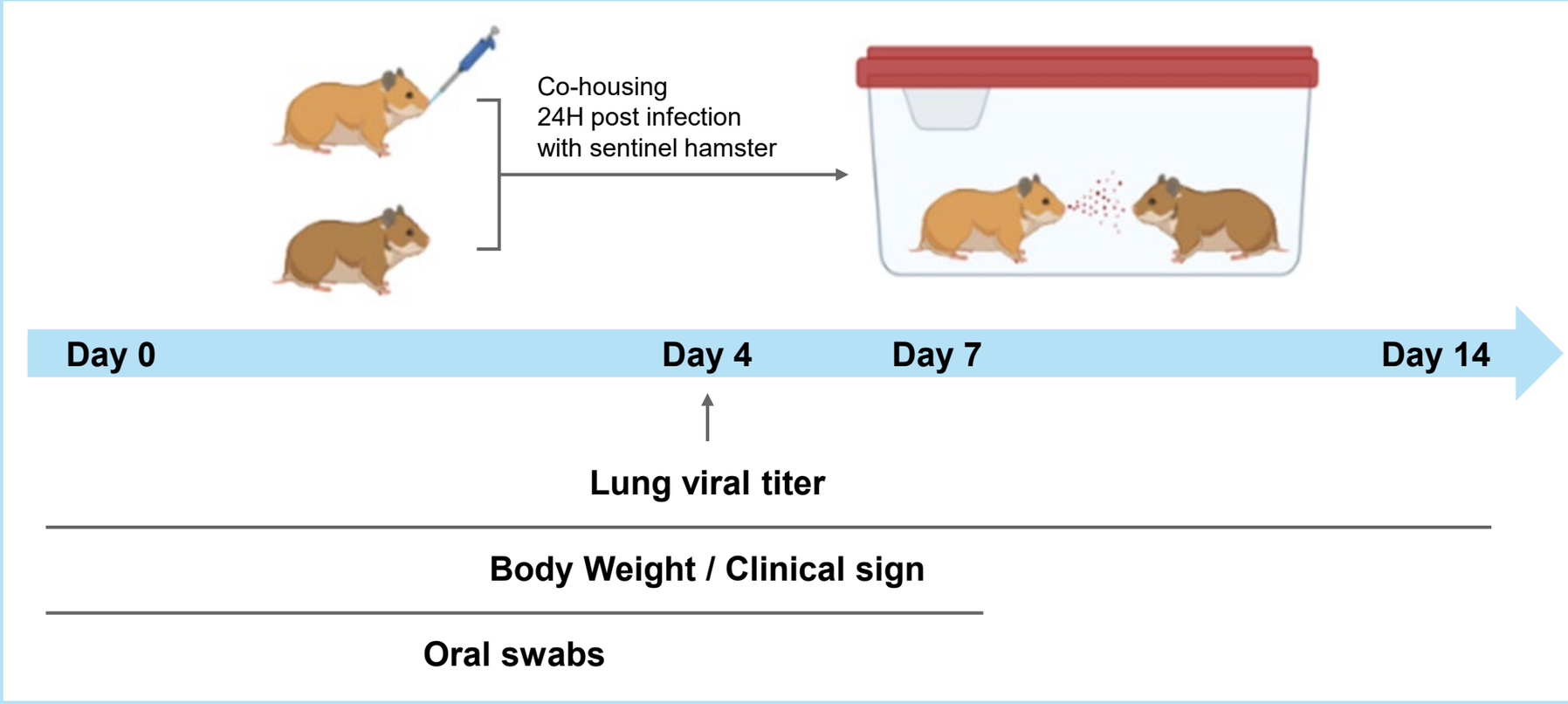


Virus Titre at Day 4



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
  - WT mouse model with virus variant



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Respiratory virus capabilities (*in vitro* and *in vivo* models)

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Hepatitis B virus capabilities

Integrated virology (Hit ID to PDC)

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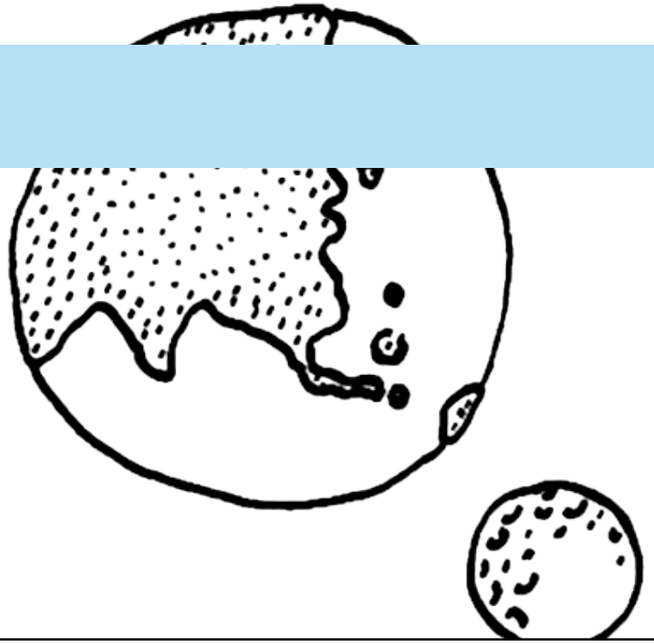


Hepatitis B Infection Model

HTS in L3 laboratory

Assay development → **Primary Screen** → **Analysis** → **Secondary Screen** → **Counter Screen** →

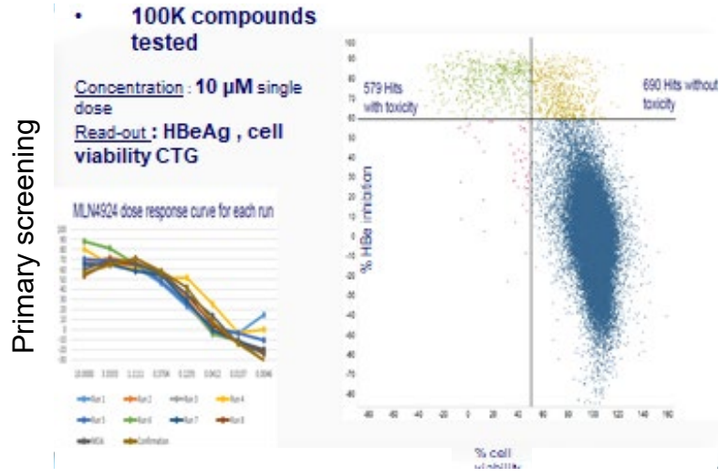
- **Highest quality chemical start points**
- **Best value**

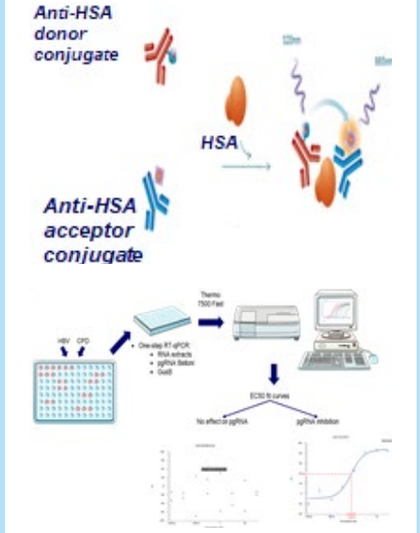
- Choice of cells
  - Primary Human Hepatocytes
  - HepaRG
  - HepG2-NTCP
- Plate format
  - 96w
  - 384w
  - 1536w
- Treatment conditions
- Readouts

Primary assay (HTRF assay)	3µM One treatment	3µM One treatment	3µM Two treatment
Signal/Background	5.26	5.63	5.59
Z'-factor	0.55	0.54	0.59
Reference compound IC_{50} (nM)	122.6	192.7	238.5
Reference threshold (%)	46.2	49.97	52.98
# Cpds tested	1,994	1,992	1,993
# Primary Hits	170	231	289
Primary hit rate (%)	2.84	3.87	4.83
# Confirmed Hits	49	74	84
Rate of confirmed hits (%)	2.46	3.71	4.21
False positive rate (%)	0.52	0.1	0.83
False negative rate (%)	0.55	0.4	0.4

Integrated Biology and Medchem Process to identify most suitable hits for progression into hit expansion

1,269 active hits





52 hits for follow-up

Molecular term	Value
3D Shape	Chiral
LogP	0.16
MW	287

HBV infections models and readouts

Available assays

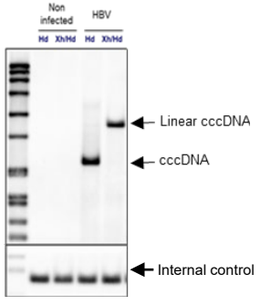
Antiviral assays

- Primary Human Hepatocytes
- HepaRG
- HepG2-NTCP
- HepaD38

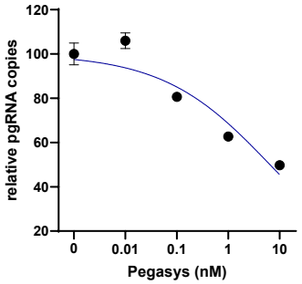
Readouts

- cccDNA
- Cellular viral DNA
- Cellular viral RNAs
- Cellular viral proteins
- Circulating DNA
- HBe/HBs release
- Cell viability
- Cytokines release

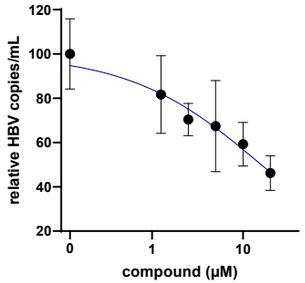
cccDNA



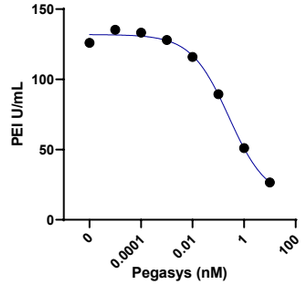
Intracellular pgRNA



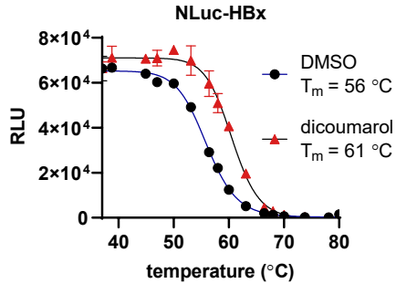
Secreted HBV DNA



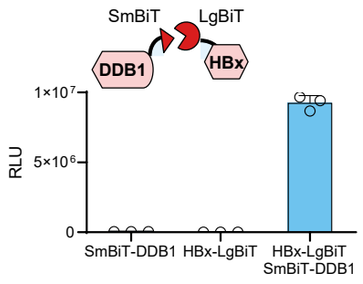
HBe release



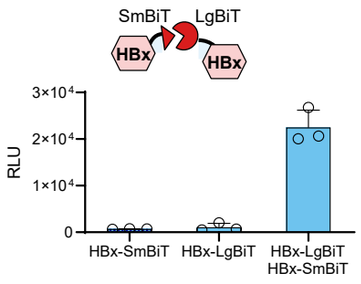
Cellular thermal shift (CTS)



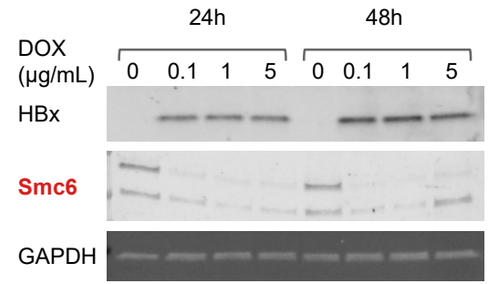
HBx DDB1 interaction



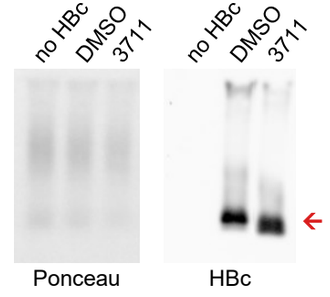
HBx dimerization



Smc6 degradation



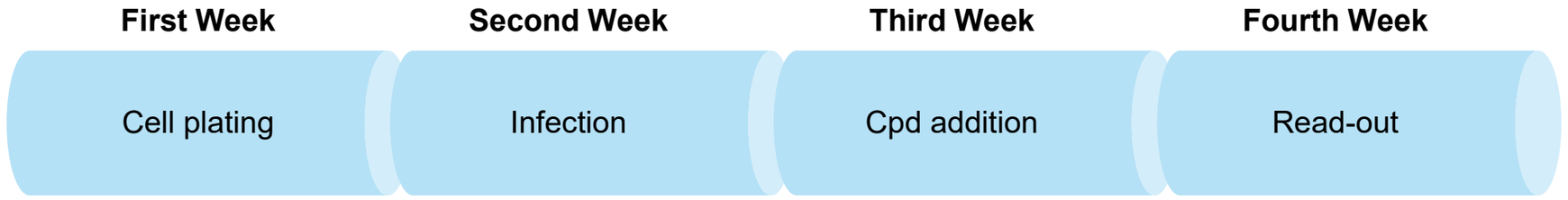
Nucleocapsid formation

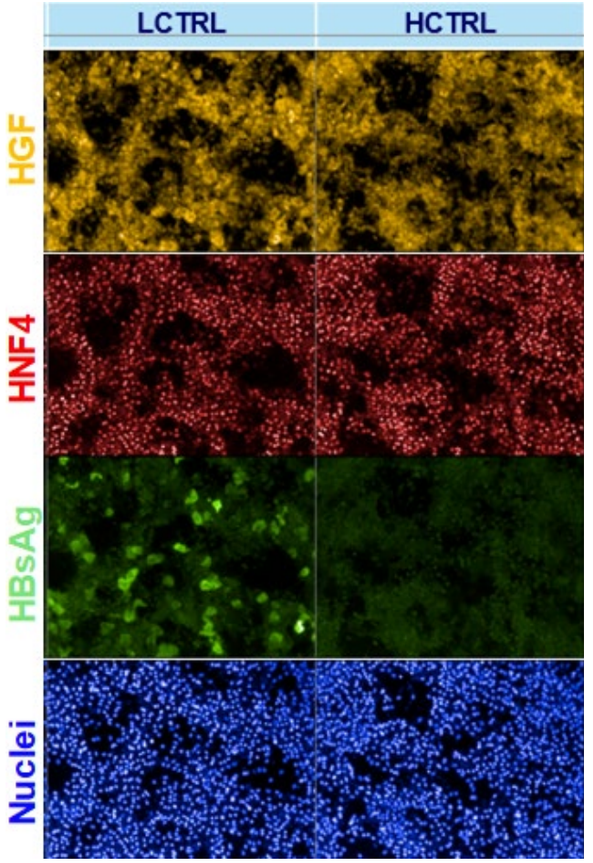


Hepatitis B infection assay

Assay principle

- High content screening assay in human hepatocytes derived from HepaRG cells infected with Hepatitis B Virus
- 4 week assay in BSL3** environment
- Readout: 3 marker + nuclear staining (Hoechst)
  - HGF (hepatocyte growth factor): cytoplasmic marker for hepatocytes
  - HNF4 (hepatocyte nuclear factor 4): nuclear marker for differentiated hepatocytes (loss of HNF4 leads to de-differentiation)
  - HBsAg (Hepatitis B surface antigen): cytoplasmic marker for HBV
- HTS with 110K small molecules resulting into ~1% "specific" confirmed hits
- Highly successful Hit ID campaign. Identified hits progressed by partner.



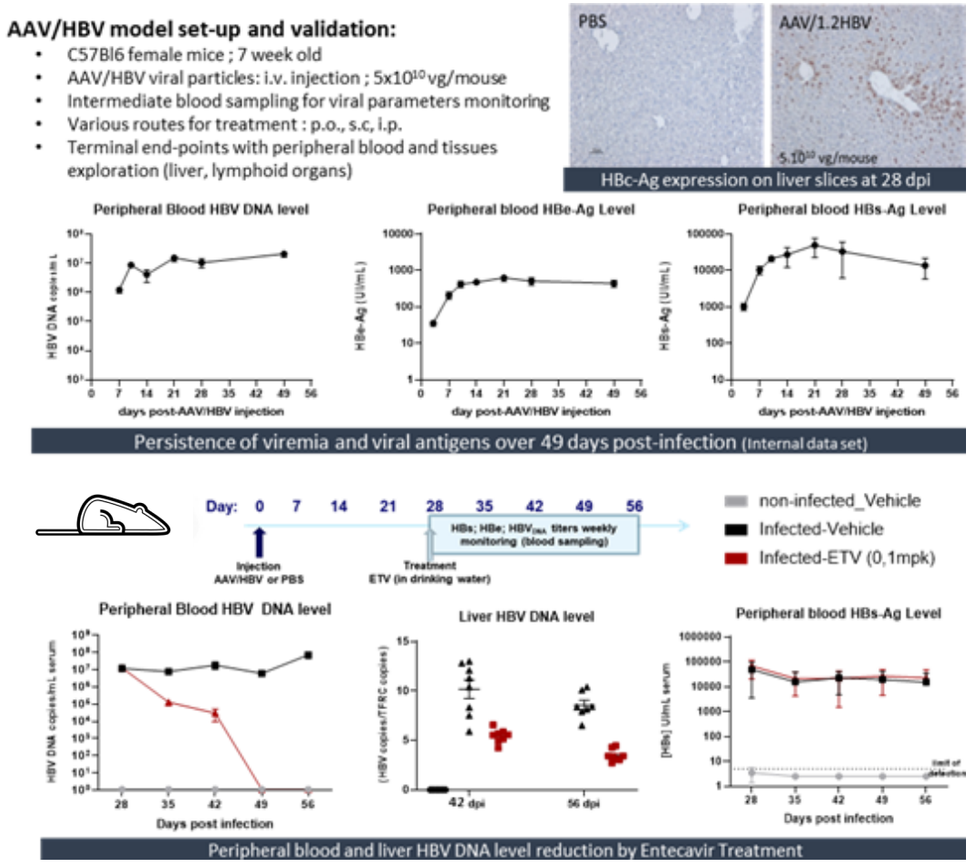


HCTRL: uninfected/DMSO = highest inhibition of HBsAg
LCTRL: infected/DMSO = lowest inhibition of HBsAg

HBV *in vivo* models

AAV/HBV mouse model

- Attributes
  - Immune competent animals transduced with HBV via AAV carrier
  - Persistent viral products over time
  - Protocol for treatment tailored for specific applications (direct antivirals or host-targeting agents)
- Readouts
  - Circulating viral DNA, RNA
  - HBeAg, HBsAg, HBcAg
  - AST/ALT
  - Anti-HBsAg, anti-HBcAg antibodies
  - Activated immune cells
  - Liver viral DNA, RNA and cccDNA



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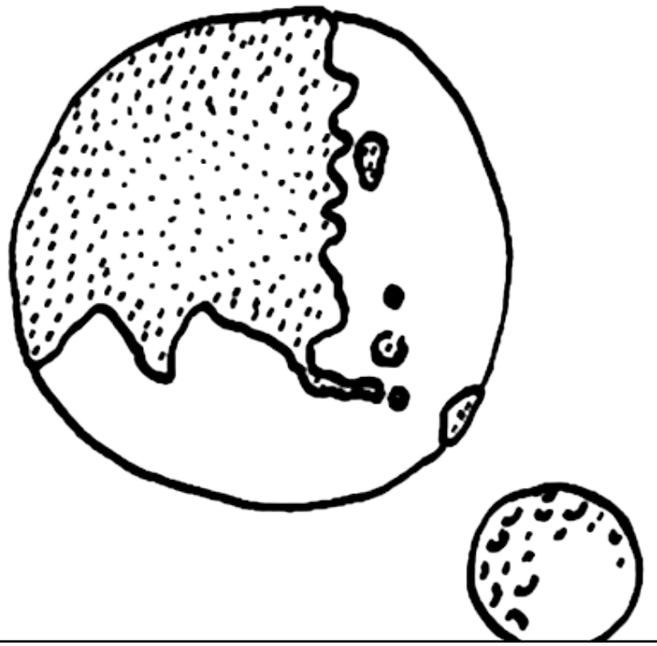


Leader in Infectious Diseases Discovery Platforms

A global partner delivering new anti-infectives

Viral infections

- Evotec is supporting NIH-led initiative Therapeutic Interventions and Vaccines (“ACTIV”)
- Evotec is leading “COVID R&D” – pre-clinical repurposing
- Building world-leading footprint in antivirals

Bacterial infections

- Partnerships to discover novel anti-biotics (GARDP, Forge, GNA-NOW, COMBINE, IMI ENABLE, WTF AMR, AMR Industry Alliance, Novo REPAIR, …)
- CARB-X funding for development of a novel broad spectrum antibiotic project
- Alliance with Liverpool School of Tropical Medicine (LSTM): SIPF, organoids and PK/PD

Global health

- Multiple programs
  - BMGF: TB Hollow Fibre Systems to model (quadruple!) drug combinations
- New opportunities in Non-TB mycobacteria (CF, etc.)
- New TB initiatives (e.g. PAN-TB, ERA4TB)

State of the art, multimodality anti-infective discovery platform and world-leading expertise

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

Evotec – A truly global footprint

ONE ID Team

Deep roots in ID and Heritage

- More than 20 years of experience in integrated drug discovery and development from target identification to clinical trials

Strong Expertise

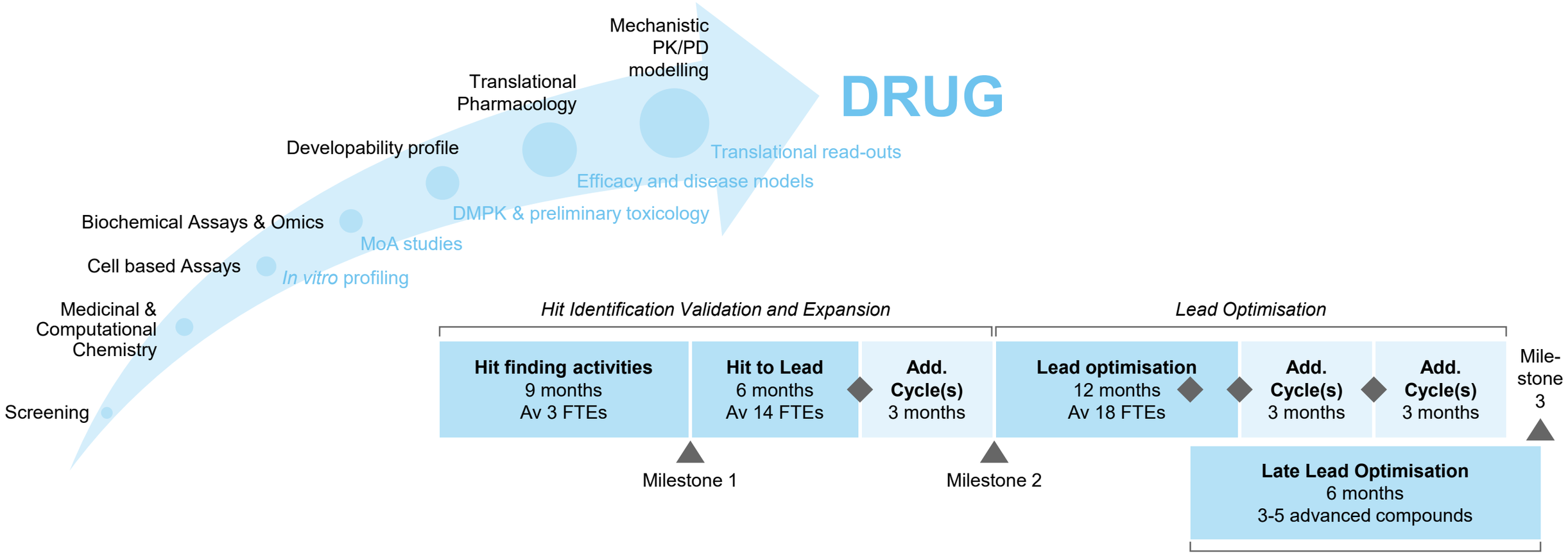
- Team of highly skilled and expert scientists
- Successful leadership of complex programs in a matrix environment
- Dedicated technologies and scientific platform

Established Relationships

- Large global network – open innovation
- Public-private partnerships
- Foundations
- National and International Institutions
- Opinion Leaders

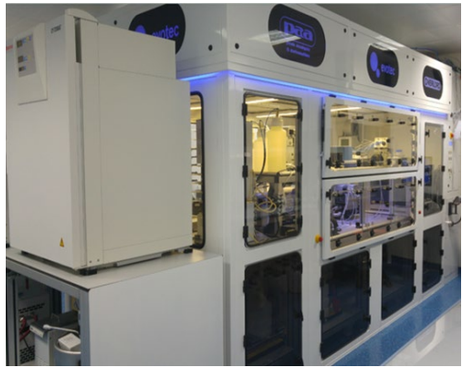
Integration of knowledge is key

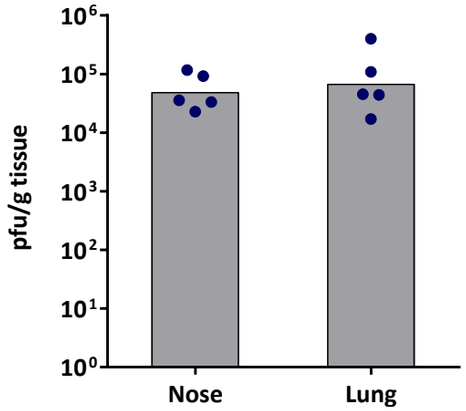
ONE ID Team

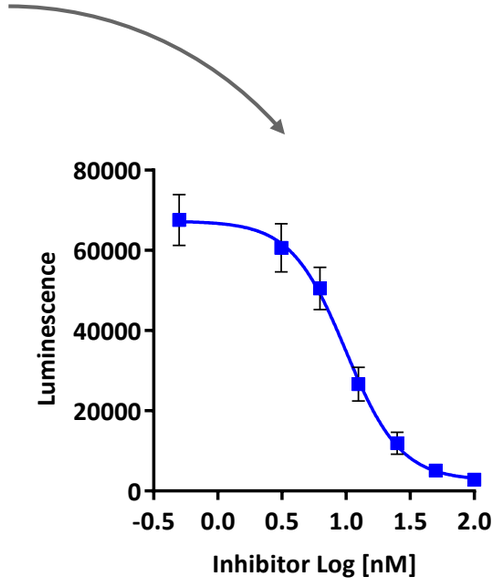


Virology: from screening to animal models

- Antiviral HTS experience from reporter based replicon readouts to infected cell assays handled in BSL2^{+}/BSL3
- Medium throughput (up to 10K compounds) in 96- and 384-well format in infected cell assays with metabolic or enzyme read-out
- Regular SAR screening for integrated programmes – antiviral vs. cytotoxicity
- MOA work e.g. resistant virus generation, order of addition effects, cell and virus strain specificity
- Employment of Evotec platforms for target identification e.g. PhotoAffinity Labelling Mass Spectrometry studies in infected and uninfected cells
- Routine PK in mouse and rat, other rodent hosts are possibility
- Development of relevant animal models
- Disease relevant expertise e.g. in respiratory area







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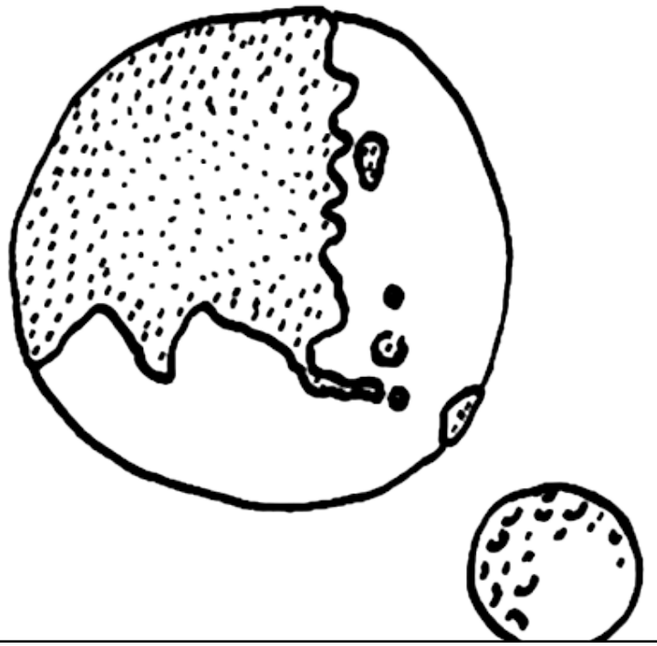


Virology at Evotec

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

Why biomarkers?

Improving success in the clinic

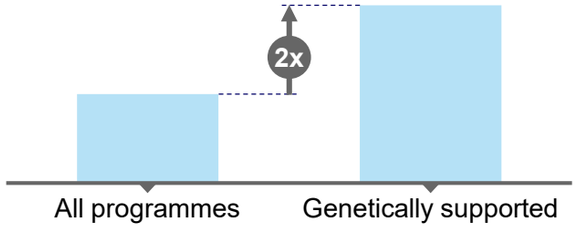
Discovery projects and clinical trials often fail due to lack of efficacy and safety

Having one or more translatable biomarkers to measure safety, stratify patients (responders/ non-responders), and ensure target engagement (PD) and efficacy (endpoint) will:

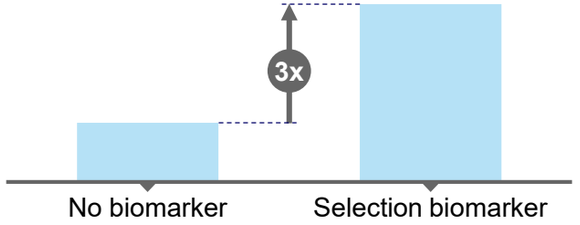
1	Improve the chance of success (better target, better drug, better design)
2	Reduce the cost of (pre)clinical development
3	Improve the chance of approval
4	Significantly increase market uptake and acceptance
5	Add value to the asset (especially if combined with CDx[3])

Precision

Human genetics supported targets[1]
% success



Biomarker based stratification[2]



[1] Margan, P. et al. Nature Rev Drug Discovery 2018 Mar 17 (3): 167-181
[2] Evotec-Bayer report "Excelling Together for the Benefit of Women Suffering from Endometriosis"
[3] CDx = companion diagnostic

Biomarker and translational research at Evotec

Partner of choice

1	**Broad target class and disease area expertise** enables Evotec to comprehensively address biomarker and translational research needs
2	Wide range of validated **primary and stem cell systems and animal models** for biomarker discovery and pre-clinical validation
3	Multiple technology platforms enabling biomarker discovery based on Evotec's **high-end genomics, transcriptomics, proteomics and metabolomics** expertise
4	Flexible solutions for **biomarker verification and validation** involving versatile immunoassays, targeted proteomics and transcriptomics
5	**Full translational capabilities** supported by *in vivo* pharmacology, imaging and immunohistochemistry platforms, and privileged access to **annotated clinical sample material**

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

#RESEARCHNEVERSTOPS

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